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Quantitative chromatographic separation of total organic phosphorus from soil extracts

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QUANTITATIVE CHROMATOGRAPHIC SEPARATION OF TOTAL ORGANIC PHOSPHORUS FROM SOIL EXTRACTS

by

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INTRODUCTION

In an investigation of the value of measurements of soil organic phosphorus mineralization during incubation for estimating the uptake of soil phosphorus by plants, Van Diest (1957) found that in many alkaline soils more than 100% of soluble inorganic phosphorus added to soil samples before incubation could be recovered at the end of the incubation period by extraction with an anion-exchange resin. The recovery of the extra phosphorus must have been caused by either enhanced solubility of the soil inorganic phosphorus or enhanced mineralization of soil organic phosphorus.

Other investigations have suggested, either directly or indirectly, that the addition of inorganic phosphate to soils caused accelerated mineralization of the soil organic phosphorus. McCall et al. (1956) found that, upon addition of soluble inorganic phosphorus to peat and muck soils, much of the organic phosphorus disappeared during a period of 4 months.

The influence of increasing rates of applied superphosphate on the mineralization of soil nitrogen in a peat soil was studied by Kaila (1958). The mineral nitrogen content of soil samples collected from a field experiment in which superphosphate had been applied annually for 35 years was found to be highest, at all depths, in the plots treated with the highest amounts of superphosphate. The mineralization of soil

nitrogen has been highly correlated with the mineralization of soil organic phosphorus (Thompson and Black, 1950); hence, the results found by Kaila can be interpreted as additional evidence for the mineralizing effect of added inorganic phosphate on soil organic phosphorus.

The influence of added inorganic phosphate on mineralization has not been studied in detail, however, because no existing analytical method is capable of precisely measuring organic phosphorus in soil extracts which contain excessive quantities of soluble inorganic phosphates. All existing methods follow the same general procedure, namely, determination of inorganic phosphorus in soil extracts before and after some treatment to change the organic phosphorus to the inorganic form, the amount of organic phosphorus being calculated from the difference. The treatment may be applied to the soil itself or to an aliquot of the extract which contains the organic phosphorus. In any solution in which inorganic phosphorus makes up a high proportion of the total phosphorus content, organic phosphorus must be determined from the relatively small difference between two numbers. Even a small error, percentagewise, in determining either number could cause a large error, percentagewise, in the difference.

The objective of this investigation was to find and develop a method capable of the accurate and precise determination of organic phosphorus in soil extracts which contain

large quantities of soluble inorganic phosphorus. The development and subsequent application of the proposed procedure could provide a better understanding of the phenomenon of phosphate-induced mineralization of soil organic phosphorus.

REVIEW OF LITERATURE

None of the existing methods for the determination of total organic phosphorus in soil extracts include a separation of the two phosphorus forms, organic and inorganic, prior to analysis. A procedure has been developed, however, which includes a separation of inorganic phosphorus from acid extracts of plant materials. The inorganic phosphorus determination utilizes a colorimetric method involving extraction of a molybdenum blue complex with isobutyl alcohol (Pons and Guthrie, 1946). The method was reported to determine inorganic phosphorus in acid extracts without interference from phytin and the carbohydrate esters of phosphoric acid (Pons et al., 1953). Whether the separation was quantitative with respect to either inorganic or organic phosphorus was not reported. It seems unlikely that all organic phosphorus compounds of plants would be insoluble in isobutyl alcohol. Furthermore, part of the isobutyl alcohol dissolves in the aqueous phase. Solubility tables list the solubility of isobutyl alcohol in water as 9.5 g. of alcohol per 100 ml. at a temperature of 18°C. (Hodgman, 1954). On the other hand, certain types of liquid-to-liquid extractions are reported to be highly selective. The solvent extraction of heteropoly acids has been used as a means of purifying the acids (Morrison and Freiser, 1957). The method of Pons and Guthrie has recently been applied to water extracts of soil (Watanabe

and Olsen, 1962). The isobutyl alcohol extraction procedure appeared to merit investigation as a possible method for the quantitative separation of the two phosphorus forms, organic and inorganic.

Inorganic phosphorus can be extracted from aqueous solutions by ether provided the solutions are strongly acidified with HCl in the presence of $FeCl_3$ (Feigl, 1949). About one half of the inorganic phosphorus is transferred from the aqueous phase into the ether. This particular liquid-to-liquid extraction procedure does not appear to warrant investigation because of the low yield of inorganic phosphorus. In addition, part of the organic phosphorus might be soluble in ether. The solubility of ether in water is over 10%, volumewise. Finally, the possibility of analytical difficulties must be considered because of the presence of ferric ions in the system.

Goring (1955) developed a procedure which separated up to 90% of the organic phosphorus compounds from soil extracts. Activated carbon was added to an acidified NaOH extract of soil, and the ingredients were mixed. The carbon and sorbed phosphorus compounds were removed from solution by filtration and were washed with 0.5 N HCl. The carbon and filter paper were then ignited at 500°C. in a muffle furnace. The phosphorus content of the ash was determined colorimetrically. Contamination by inorganic phosphorus, reported to be less

than 1%, was determined by tagging the inorganic phosphate content of soil extracts with radioactive phosphorus and then measuring the radioactivity of the ashed carbon.

Activated carbon has had previous application in soil chemistry as a decolorizer of soil extracts prior to inorganic phosphorus analysis (Yuen and Pollard, 1953; Olsen et al., 1954). The sorptive capacity of carbon for a variety of organic substances has been established (Deitz, 1956).

The sorptive powers of activated carbon for specific compounds have been attributed to van der Waal's forces at the solid:liquid interface, to the existence of exchange sites, and to carbon-to-carbon bond spacing (Williams, 1947; Hassler, 1951). The ability of carbon to sorb up to 90% of the organic phosphorus and less than 1% of the inorganic phosphorus from acidic soil extracts as reported by Goring suggests that the effective bonding forces are predominantly non-ionic, possibly carbon-to-carbon, i.e., between carbon atoms on the surfaces of the activated carbon and the carbon atoms of the organic phosphorus compounds.

Although Goring's procedure failed to produce a quantitative separation of organic phosphorus, the results indicated that further investigation and development of the procedure were justified. The work of Smith and Clark (1952), who used chromatographic columns prepared with a weak-base anion resin to separate specific soil organic phosphorus compounds, sug-

gested the use of a chromatographic technique. The use of carbon as the solid adsorbent in chromatographic columns might improve the separation of organic phosphorus because of the repeated opportunity for sorption on fresh carbon surfaces. The one component present in all organic phosphorus compounds and, by definition, lacking in all inorganic phosphorus compounds is organic carbon. If the chromatographic adsorbent were specific for organic carbon, and if the sorbed compounds were tightly retained, the desired separation could be achieved.

PRELIMINARY DEVELOPMENTS

Using the difference between the total and inorganic phosphorus content of solutions to determine the organic phosphorus provides values of relatively low precision where the solutions contain much inorganic phosphorus. Much better precision could be obtained if a direct analysis could be made for organic phosphorus or if most of the inorganic phosphorus could be removed before the method of difference is applied. Because no way is known to make a direct analysis for the total organic phosphorus of soil extracts in the presence of inorganic phosphorus, the physical separation of the organic and inorganic phosphorus appears to be a prerequisite to the desired direct determination. The separation might be accomplished in two ways. First, the inorganic phosphorus might be removed from the soil extract, leaving behind the organic phosphorus. A direct analysis for organic phosphorus could then be made by igniting it and measuring the inorganic phosphorus thus produced. Second, the organic phosphorus might be removed from the soil extract, leaving behind the inorganic phosphorus. A direct analysis for the total organic phosphorus could then be made in the manner just mentioned.

Separation of Inorganic Phosphorus from Aqueous Solutions

Because of the isobutyl alcohol extraction procedure of Pons and Guthrie (1946), the convenient approach to the devel-

opment of a separation procedure appeared to be from the standpoint of the separation of inorganic phosphorus from solution, leaving behind the organic phosphorus. The procedure of Pons and Guthrie includes the separation of inorganic phosphorus from acid extracts of plant material by means of a liquid-to-liquid extraction of the molybdophosphoric acid complex from the aqueous phase into isobutyl alcohol. The blue color of the reduced complex is developed and measured in the alcohol phase. One of the advantages of the method is the elimination of possible interference by colored organic matter in the aqueous phase which apparently is not extracted into the alcohol.

The thoroughness of the extraction step was investigated using inorganic phosphorus standards only. The molybdophosphoric acid complex was formed in the aqueous phase and then extracted into the isobutyl alcohol. As a test of the completeness of the extraction, the aqueous phase was examined. The aqueous layer was removed from the separatory funnel and subjected to the perchloric acid digestion treatment as normally used for the digestion of organic phosphorus compounds. An aliquot of the digest was then placed in a colorimeter tube for phosphorus determination by the usual colorimetric test. Upon neutralization with alkali, before the addition of molybdate reagent, the digest became turbid and developed a blue color.

Apparently some of the molybdate remained in the aqueous phase and was altered by the digestion treatment with perchloric acid in such a manner that a blue color developed, irrespective of the presence of phosphorus. The difficulty was thought at first to result from the heating of the aqueous phase during digestion. Van Wazer (1958) reported that heat could cause the formation of a molybdomolybdate complex which produced the same blue color as the molybdophosphate complex.

An attempt was made to circumvent the difficulty by digesting the aqueous phase at room temperature using a 30% solution of hydrogen peroxide which had been specially purified according to the method of Chang and Jackson (1956). The modification was not successful. During digestion the solution became turbid and blue. Further study suggested that the color development may have been caused by formation of colloidal molybdenum blues as a result of oxidation-reduction reactions with the peroxide (Moeller, 1952).

A series of molybdate reagents then was prepared with varying concentrations of molybdate with the objective of reducing the amount of molybdate which remained in the aqueous phase after the alcohol extraction. The results were again unsatisfactory. The isobutyl alcohol failed to extract inorganic phosphorus from aqueous solutions that had been treated with the more dilute molybdate solutions. Apparently a high

ratio of molybdate to phosphate is required for the formation of the molybdophosphate complex. The problem could not be resolved, and no further tests with the isobutyl alcohol extraction were performed.

Separation of Organic Phosphorus from Soil Extracts

Goring's method

The problem of finding a means of separating the two forms of soil phosphorus, organic and inorganic, was next approached from the standpoint of removing all the organic phosphorus from the soil extract, leaving behind all the inorganic phosphorus. The method developed by Goring (1955) for the separation of organic phosphorus from soil extracts by sorption on activated carbon was investigated. Since much of the work reported in this thesis was influenced by Goring's procedure, a brief description of that procedure will be given.

The soil sample, previously treated with N HCl, is extracted twice with 0.5 N NaOH, first at room temperature and then at 90°C. The two alkali extracts are combined and mixed, and the suspended material is allowed to settle. One hundred ml. of the supernatant of the NaOH extract are placed in an Erlenmeyer flask which contains 8 ml. of concentrated HCl (this gives a solution of 0.5 N HCl) and 0.25 g. of activated carbon. The ingredients are mixed for 1 hour. A second and

third addition of carbon are made with 1-hour periods of shaking. The mixture is filtered, and the filter paper and carbon are washed with 0.5 N HCl. The carbon and filter paper are transferred to a 50-ml. beaker, treated with NH_4OH and $Mg(NO_3)_2$, evaporated to dryness on a steam plate, and ignited at 500°C. for 16 hours. The ash is taken up with N HCl and filtered into a 50-ml. volumetric flask. The phosphorus content is determined colorimetrically.

Goring reported that from 85 to 90% of the organic phosphorus present in the supernatant of the NaOH extract was separated by the method with contamination of less than 1% by the inorganic phosphorus. Goring reported also that the HCl extract was not used because it contained less than 5% of the soil organic phosphorus and over 50% of the inorganic phosphorus. The carbon (U.S.P. powder) was thoroughly washed with N HCl prior to use to remove traces of inorganic phosphorus.

The procedure described above, in which the carbon is added directly to the solution, shaken, and then removed by filtration, will be referred to hereafter as "batch extraction".

Batch extractions with carbon

The method of batch extraction using activated carbon was investigated in some detail with the objective of increasing

the efficiency of the separation. Several brands of activated carbon were subjected to different purification treatments in an attempt to reduce to a low level the phosphorus content of the carbon without reducing the sorptive capacity for organic phosphorus. Five-pound lots of each brand were placed in concentrated HCl and heated overnight on a steam plate. The carbon was removed from the acid by filtration, washed with water, and then oven-dried. In a similar manner the acid-washed carbon was then washed successively with 95% ethanol, 6 N NH_4OH, and concentrated HCl. These treatments reduced the phosphorus content of the carbons. The phosphorus content of one brand, Norit-A, was reduced from 17 to 1 ppm., and the iron content was reduced from 100 to 25 ppm.

An electrodialysis apparatus was constructed out of Plexiglas acrylic plastic according to a design of Löddesöl (1932). Large batches of the purified carbon were electrodialyzed for periods of up to 3 weeks under conditions suggested by Bradfield (1927). The results of the electrodialysis were not satisfactory. The phosphorus contents were not significantly reduced; and, with some brands of carbon, the sorptive capacity for organic phosphorus was diminished.

The failure of carbon to sorb inorganic phosphorus from acidic solutions was verified by adding carbon to standard solutions of inorganic phosphorus which had been prepared in water and in HCl of varying acid strength. The mixtures were

shaken for 1 hour, and the carbon was precipitated by centrifugation. The supernatants were analyzed for inorganic phosphorus. All the inorganic phosphorus was recovered in 0.5 N HCl, about 75% was recovered in 0.01 N HCl, and only about 10% was recovered in water solutions.

Several brands of charcoal and carbon were compared in a test of sorptive tendencies for organic phosphorus: Darco G-60 "for soils", Carbon Black G, charcoal made from cane sugar, Ultrasorb made by Norit, and Norit-A decolorizing carbon. Standard solutions of sodium phytate and ribonucleic acid were prepared as the sources of the organic phosphorus. Sugar charcoal, initially preferred because of its inherent low phosphorus content, sorbed the least organic phosphorus. Both Norit-A and Darco G-60 "for soils" sorbed organic phosphorus most effectively. Norit-A was selected for all further investigations because of its lower phosphorus content.

The batch extraction procedure was applied to soil extracts with and without added inorganic phosphorus standards. The soil extracts were obtained by the method of Mehta et al. (1954), which employs concentrated HCl and 0.5 N NaOH as the extractants. In general, the recovery of organic phosphorus on the carbon was poor--seldom more than 50%. Increasing the amount of added carbon resulted in increased sorption of the organic phosphorus; however, the advantage of increased sorption was somewhat offset by the disadvantage of the increased

content of phosphorus in the control. The efficiency of the sorption was improved to about 90% by using only the alkali extracts of soils which had been pretreated with normal acid. The alkali extracts contained a high concentration of organic phosphorus in relation to inorganic phosphorus.

Retention of inorganic phosphorus by the carbon, relatively unimportant from the alkali extracts, became an important consideration whenever solutions were used that contained a large quantity of inorganic phosphorus. To reduce the retention of inorganic phosphorus the carbon was thoroughly washed with 0.5 N HCl. But with more intensive washing some of the organic phosphorus, initially sorbed on the carbon, was desorbed and washed back into the solution. Apparently, part of the organic phosphorus was only weakly sorbed onto the carbon. Ultimately, it was concluded that the method of batch extraction could not be adapted for the quantitative separation of total organic phosphorus from soil extracts.

Chromatographic carbon columns

To improve the effectiveness of the separation of organic phosphorus from soil extracts by sorption onto activated carbon the carbon was next used as the solid adsorbent in chromatographic columns. Because the reaction mechanism of chromatographic separation with carbon columns was the same as for the batch extraction with carbon, some of the information

obtained from the experiments with the batch extraction method was assumed to be valid for column chromatography. The carbon brand Norit-A, found to be superior to other brands in the batch extraction experiments, was assumed to be superior for use in the chromatographic columns also. The purification treatments using concentrated acid and alkali and 95% alcohol were continued without re-examination. All extracts and eluants used in the chromatographic columns were prepared in 0.5 N HCl, the acidity used by Goring.

In the initial experiments, adsorption columns were prepared using 1 or 2 g. of carbon. The carbon bed was supported on a glass wool pad in a column consisting of a 50-ml. buret. A vacuum flask, attached to the tip of the buret, served to collect the eluate. After addition of the phosphorus solution to the column, a vacuum was applied to the collection flask to increase the rate of elution. After filtration of the solution, the column and carbon bed were washed with 0.5 N HCl. The acid eluate was removed for analysis of total and inorganic phosphorus. Although inorganic phosphorus was readily eluted from the column by the acid, some organic phosphorus was also recovered in the eluate. The results indicated the need for larger carbon beds.

The 50-ml. burets were used as chromatographic columns in another experiment. Each column was prepared with 24 g. of carbon, forming an adsorbing bed over 60 cm. in height.

Soil extract with and without added inorganic phosphorus was added to the column and leached through the carbon bed. The column walls and carbon bed were then washed with 0.5 N HCl. The elution process was periodically halted to permit removal of the eluate for later analysis. In this manner 10 fractions of eluate were individually collected and analyzed for total and inorganic phosphorus.

The results of the analyses showed that inorganic phosphorus from soil extract and from inorganic phosphate standards was readily eluted from the carbon by 0.5 N HCl. The appearance in the eluate of the front boundary of inorganic phosphorus was sharp and easily detected. The rear boundary was less sharply defined; there was some tendency toward "tailing off". All the inorganic phosphorus was recovered in the eluate. No significant amount of organic phosphorus was detected.

The separation of the two phosphorus forms, organic and inorganic, by means of the chromatographic carbon columns appeared to be satisfactory. The next problem was to find some means of removing the sorbed organic phosphorus from the carbon. A review of the literature provided little encouragement. Many sorptive reactions involving carbon appear to be irreversible. According to Strain's classification (Williams, 1947), activated carbon is one of the most active solid adsorbents. Nevertheless, several solvents were tested:

dilute alkali, strong acid, and several combinations of pyridine, alcohol, and dilute organic acids. None of the solvents or solvent mixtures was effective in removing the organic phosphorus sorbed on the carbon.

Since the sorbed organic phosphorus could not be recovered from the carbon, the chromatographic columns were redesigned to permit extrusion of the carbon bed after completion of the leaching and eluting processes. The carbon (and sorbed organic phosphorus) was ignited in a muffle furnace, and the inorganic phosphorus content in the ash was determined colorimetrically. The value for sorbed organic phosphorus was then calculated by correcting the total phosphorus content in the ash for the phosphorus content in the carbon itself.

MATERIALS AND METHODS

Soils

The soils used in this study were collected by Van Diest (1957). The organic and inorganic phosphorus values, determined by the method of Mehta *et al.* (1954), and the pH values for the soils are listed in Table 1. The soil samples were ground to pass a 60-mesh screen prior to extraction.

Table 1. Sample number and soil type of samples employed, together with measurements of pH and of extractable organic and inorganic phosphorus - the data are from Van Diest (1957)

Soil number	Soil type	pH	Phosphorus extracted by method of Mehta *et al.* (1954), µg. per g. of soil	
			Organic	Inorganic
F-2852	Alcaton silt loam	7.9	77	731
F-2868	Harpster silty clay loam	7.5	550	614
F-2872	Harpster silty clay loam	7.5	375	369
F-2882	Ida silt loam	7.5	237	491
F-2885	Loess	8.3	5	735
F-2887	Nicollet loam	6.4	267	189
F-2890	Onawa silty clay loam	7.8	82	646
F-2902	Shelby loam	6.0	171	117
F-2915	Webster silty clay loam	7.1	245	587

Purification of Carbon

Norit-A decolorizing carbon, supplied by the Fisher Scientific Company, Fair Lawn, New Jersey, was placed in concentrated HCl, and the mixture was heated overnight on a steam plate. The carbon was removed from the acid by filtration, washed with water, and oven-dried. In a similar manner the acid-washed carbon was washed with 95% alcohol, 6 N NH_4OH, and concentrated HCl. The phosphorus content of the purified carbon was 1 ppm.

Preparation of the Columns

Except as indicated specifically, the chromatographic columns consisted of 40-cm. sections of Pyrex tubing having an inside diameter of 1.6 cm. The lower end of the column was stoppered with a No. 2 rubber stopper having a single drain tube. The drain tube was connected to a 500-ml. vacuum flask which served to collect the eluate. A pad of glass wool was inserted into the column to form a support for the adsorbing bed. A suspension of diatomite filter-aid (Celite, supplied by the Fisher Scientific Company, 2850 S. Jefferson, St. Louis, Missouri) in 0.5 N HCl was added to the column and allowed to settle. Carbon suspended in 0.5 N HCl was next added to the column. A compact bed of carbon was formed on the layer of filter-aid by applying vacuum to the collection flask as the carbon settled. A 4-g. amount of carbon

formed an adsorbing bed about 5 cm. in height. Excess acid was drained from the column, leaving enough to keep the adsorbing bed barely submerged.

Chromatographic Procedure

An aliquot of soil extract, or inorganic phosphorus solution, was added to the chromatographic column, followed immediately by the addition of a small amount of suspended filter-aid. (The solid organic matter of the soil extract and the filter-aid flocculated into large porous aggregates which settled onto the carbon bed. Without the filter-aid the solid organic matter clogged the pores in the carbon bed and made elution extremely slow.) A vacuum was applied to the collection flask to hasten the elution process. After the solution had infiltrated to the top of the carbon bed, the column walls were washed several times with 0.5 N HCl, and then an overhead reservoir was attached to the column to supply the eluant, 0.5 N HCl.

Ignition of the Carbon

After the elution process was completed, the column was dismantled, and the carbon bed was extruded into a 50-ml. beaker. The contents were treated with 5 ml. of 6 N NH_4OH and 1 ml. of a 10% solution of $Mg(NO_3)_2$. The mixture was evaporated to dryness on a steam plate and then ignited at

500° to 520°C. in a muffle furnace.

After ignition of the carbon was complete, the beaker was removed and cooled. To the beaker were added 20 ml. of N HCl, and the beaker was heated for 10 minutes on a steam plate. The solution (and suspended filter-aid) was transferred to a 50-ml. volumetric flask, diluted to volume with water, and thoroughly mixed. The filter-aid was allowed to settle overnight before aliquots were taken for phosphorus analysis. Phosphorus was determined colorimetrically by the method of Dickman and Bray (1940) as modified by Legg and Black (1955).

Extraction and Determination of Soil Organic Phosphorus

In the early stages of development of the chromatographic procedure the soil extracts were obtained by the method of Mehta et al. (1954), in which soils were subjected to successive extractions with concentrated HCl, 0.5 N NaOH at room temperature, and 0.5 N NaOH at 90°C. The combined extracts formed a solution which was 0.5 N in HCl and 0.25 N in NaCl.

Total phosphorus was determined in aliquots which had been subjected to digestion with perchloric acid. Inorganic phosphorus was determined in untreated aliquots of the soil extract. The difference between total and inorganic phosphorus was taken to represent organic phosphorus.

EXPERIMENTS

Introductory Remarks

The initial experiments in the development of the chromatographic column separation were designed to provide information which might be used to answer the following fundamental questions: 1) Can a single set of conditions be found under which organic phosphorus is sorbed quantitatively and inorganic phosphorus is eluted quantitatively? 2) What is the interaction between the phosphorus composition of the solution and the conditions required for quantitative sorption of organic phosphorus and quantitative elution of inorganic phosphorus? 3) What is the range of conditions within which organic phosphorus is sorbed quantitatively and inorganic phosphorus is eluted quantitatively?

Elution of Inorganic Phosphorus from Chromatographic Columns Containing 4 g. of Carbon

Introduction

The purpose of the first experiment was to investigate the effect of adding large quantities of inorganic phosphorus to the chromatographic columns. The elution pattern of the inorganic phosphorus from the columns was studied. As a result of adding only inorganic phosphorus to the columns, the retention of inorganic phosphorus by the carbon could be

investigated without concern about the problem of differentiating between organic and inorganic phosphorus sorbed by the carbon.

Procedure

Inorganic phosphorus solutions were prepared with KH_2PO_4 in 0.5 N HCl in concentrations ranging up to 10^6 µg. of phosphorus per 25 ml. Chromatographic columns were prepared with 4 g. of carbon, which formed an adsorbent bed approximately 5 cm. in height. Twenty-five milliliter aliquots of the inorganic phosphorus solution were added to duplicate columns, and the columns were eluted with 0.5 N HCl. The eluates were collected in increments, and the increments were analyzed individually for inorganic phosphorus. After the elution process was completed, the carbon was removed and analyzed for phosphorus. The experiment was repeated three times using different total volumes of eluate, 50, 100, and 200 ml.

Results and discussion

The results of the experiment are presented in Table 2 and Table 3 The first table shows the elution pattern of inorganic phosphorus from the chromatographic columns. The second table shows the retention of inorganic phosphorus on the carbon.

The experimental results indicate that small amounts of

Table 2. Recovery of inorganic phosphorus in increments of eluate from columns treated with different quantities of inorganic phosphorus and eluted with 0.5 N HCl

Added inorganic phosphorus, μg.	Inorganic phosphorus recovered in indicated increment of eluate, μg.								
	Total eluate = 50 ml.		Total eluate = 100 ml.			Total eluate = 200 ml.			
	0 to 25 ml.	25 to 50 ml.	0 to 50 ml.	50 to 75 ml.	75 to 100 ml.	0 to 50 ml.	50 to 100 ml.	100 to 150 ml.	150 to 200 ml.
0	0.2	0.0	0.0	0.3	0.0	0.0	0.6	0.0	0.0
100	97.5	1.5	102	0.2	0.0	101	0.0	0.0	0.0
1,000	970	4.0	985	0.5	0.9	983	1.5	0.2	0.6
100,000	97,500	520	100,000	53.0	7.2	100,000	68.0	11.3	4.9
1,000,000	970,000	6,500	990,000	166	20.9	992,000	245	49.0	21.2

Table 3. Retention of inorganic phosphorus on 4 g. of carbon from columns treated with different quantities of inorganic phosphorus and eluted with 0.5 N HCl

Added inorganic phosphorus, µg.	Inorganic phosphorus retained on carbon after elution with indicated volume of 0.5 N HCl, µg.		
	50 ml. HCl	100 ml. HCl	200 ml. HCl
100	0.20	0.00	0.20
1,000	0.70	0.90	0.60
100,000	35.6	17.8	11.2
1,000,000	129	55.6	33.9

inorganic phosphorus can be almost quantitatively eluted from the chromatographic columns by 0.5 N HCl. Significant quantities of inorganic phosphorus were retained in the columns which had received the larger applications of inorganic phosphorus, even after elution with up to 200 ml. of 0.5 N HCl. In general, as the addition of inorganic phosphorus was increased, the quantity retained on the carbon also increased. As the volume of eluant was increased, the quantity of inorganic phosphorus retained on the carbon decreased.

Effect of Delayed Leaching on the Elution of Inorganic Phosphorus from Chromatographic Columns

Introduction

In the previous experiment small but significant quantities of inorganic phosphorus were retained by the carbon

from columns which had received the larger quantities of inorganic phosphorus. Since the objective was to develop a procedure capable of the determination of organic phosphorus in soil extracts which contained a high concentration of inorganic phosphorus, it was imperative that the retention of inorganic phosphorus by the carbon be reduced to a very low level. This experiment was conducted to learn if variations in the elution procedure affected the retention of inorganic phosphorus.

Procedure

An inorganic phosphorus solution was prepared with KH_2PO_4 in 0.5 N HCl having a concentration of 10^6 µg. of phosphorus per 25 ml. Duplicate chromatographic columns which had been prepared with 4 g. of carbon received 25-ml. aliquots of the phosphorus solution. The inorganic phosphorus solution was drawn into the carbon bed in all columns. In half of the columns the elution process with HCl was begun immediately. In the other columns the phosphorus solution remained in contact with the carbon for 13 hours before the elution process was started. After 75% of the eluant had leached through the columns, the elution process was interrupted for 18 hours in half of the columns. The eluates were collected in 50-ml. fractions from all columns and analyzed for inorganic phosphorus. The phosphorus content of the carbon was also deter-

mined.

Results and discussion

The results of the experiment are presented in Table 4. The 18-hour delay in the final stages of the elution process significantly reduced the amount of inorganic phosphorus retained on the carbon. Prolonging the time of contact between the phosphorus solution and the carbon increased the amount of inorganic phosphorus retained in the column. The effect of the delay in the elution process did not overcome the effect of prolonged contact between the inorganic phosphorus and the carbon when both delays were included in the treatment.

The experimental results might be explained by advancing an hypothesis concerning the physical structure of activated carbon. If carbon exists as porous particles or aggregates, inorganic phosphorus could diffuse into the carbon and become trapped. Diffusion of the phosphorus into carbon would be enhanced by a prolonged period of contact between the phosphorus solution and the carbon. Delays in the final stages of the elution process would allow phosphorus to diffuse out of the carbon, resulting in a reduction of retained inorganic phosphorus.

The results of this experiment suggest that if elution were started immediately after addition of the phosphorus,

Table 4. Recovery of inorganic phosphorus in increments of 0.5 N HCl eluate and in ashed carbon from columns subjected to different elution procedures; all columns received 10^6 µg. of inorganic phosphorus

Elution procedure	Inorganic phosphorus in indicated increment of eluate, µg.				Inorganic phosphorus retained by carbon, µg.
	0 to 50 ml.	50 to 100 ml.	100 to 150 ml.	150 to 200 ml.	
Normal, without delays	1,040,000	288	60.0	21.0	31.0
Delay of 18 hours after elution of 150 ml. of HCl	1,040,000	214	42.0	46.0	7.0
Delay of 13 hours before start of elution	1,000,000	575	152	86.0	315
Delay of 13 hours before start of elution plus delay of 18 hours after elution of 150 ml. of HCl	1,040,000	706	220	377	77.0

the inclusion of several delays in the latter part of the elution process might reduce the retention of inorganic phosphorus to negligible values, even with extremely large additions such as those employed experimentally. Probably several days would be needed to accomplish the desired result.

Effect of Delayed Leaching on Soil Extract with and without Added Inorganic Phosphorus

Introduction

The effect of a delay in the final stages of the elution process on the retention of inorganic phosphorus by carbon was shown in the previous experiment. Because the general objective of the work was to see if quantitative sorption of organic phosphorus could be obtained concurrently with quantitative elution of inorganic phosphorus, an experiment was carried out to investigate the effect of delays in elution on the retention of organic phosphorus by the carbon. At the same time, treatments were included to investigate the possible effect of a large quantity of inorganic phosphorus on the retention of organic phosphorus.

Procedure

Samples of Harpster silty clay loam F-2868 were extracted with concentrated HCl and 0.5 N NaOH according to the method of Mehta *et al.* (1954). Aliquots of the soil extract and

aliquots of an inorganic phosphorus solution which contained 10^6 µg. of phosphorus per aliquot were added, separately or in combination, to duplicate chromatographic columns which had been prepared with 4 g. of carbon. All columns were eluted with a total volume of 250 ml. of 0.5 N HCl. The elution process was conducted without interruption in half of the columns. In the other columns the elution process was interrupted twice, once for 17 hours after 150 ml. of HCl had leached through the columns and again for 17 hours after 200 ml. of HCl had leached through the columns. The final two 50-ml. increments of eluate were collected separately and analyzed for inorganic phosphorus. The phosphorus content of the carbon was also determined.

Results and discussion

The results of the experiment are given in Table 5. The organic phosphorus content of the soil extract was 36.8 µg. of phosphorus per 25 ml. as determined by the method of difference between total and inorganic phosphorus. In the columns which had received only soil extract, the amount of phosphorus sorbed on the carbon was not affected by the two 17-hour delays in elution. In the columns which had received only inorganic phosphorus solution the amount of phosphorus sorbed on the carbon was reduced from 18.5 to 4.3 µg. of phosphorus per column by including the two 17-hour delays. The

Table 5. Effect of delayed leaching on chromatography of phosphorus added to carbon columns as soil extract, with and without added inorganic phosphorus, and eluted with 250 ml. of 0.5 N HCl

Phosphorus added to columns, µg.				Inorganic phosphorus in indicated increment of eluate, µg.		
Organic from soil extract	Inorganic: From soil extract	Inorganic: From inorganic phosphorus solution	No. of delays during elution	150 to 200 ml.	200 to 250 ml.	Increase in phosphorus content of carbon, µg.
36.8	39.7	0	0	0.5	0.0	33.6
0	0	10^6	0	43.0	9.3	18.5
36.8	39.7	10^6	0	68.6	12.2	54.1
36.8	39.7	0	2	0.0	0.2	33.6
0	0	10^6	2	60.1	4.3	4.3
36.8	39.7	10^6	2	273	43.1	38.2

phosphorus content of carbon from columns which had received both soil extract and inorganic phosphorus was about equal to the sum of the phosphorus contents of carbon from columns which had received soil extract and from columns which had received inorganic phosphorus.

The results indicate that the sorption of organic phosphorus on the carbon was virtually independent of the concentration of inorganic phosphorus in the solution. According to this experiment, therefore, delays in the latter part of the elution process are beneficial in the separation of organic and inorganic phosphorus because they enhance removal of inorganic phosphorus but are without effect on retention of organic phosphorus.

The desired quantitative elution of inorganic phosphorus was not obtained with the two delays during the elution employed in this experiment. Probably two or three more delays would be required before the residual inorganic phosphorus would be low enough to disregard.

The value for organic phosphorus as determined by the chromatographic columns was only 91% of the value as determined by the method of difference. The apparent incomplete recovery of added organic phosphorus in the carbon is not of particular significance in interpretation of the results of this experiment for reasons that will be pointed out later.

Effect of Volume and Concentration of NaOH Extracts of Soil in Columns Containing 10 g. of Carbon

Introduction

Some information was provided in the first three experiments on the elution of inorganic phosphorus from chromatographic columns. The preceding experiment showed a constant value for organic phosphorus by the chromatographic method, but the value was lower than the organic phosphorus that was added to the columns, according to analyses by the method of difference. This discrepancy raised the possibility that the constant values obtained for organic phosphorus by the chromatographic method represented merely a constant proportion of the total organic phosphorus and not the true total. Accordingly, an experiment was designed to provide information on the sorption of organic phosphorus from soil extracts. As a means of providing a relatively critical test, an extract high in organic phosphorus and low in inorganic phosphorus was employed.

Procedure

One-gram samples of Harpster silty clay loam F-2868, previously treated with N HCl, were extracted with 90 ml. of 0.5 N NaOH at room temperature for 1 hour and with a second 90 ml. of 0.5 N NaOH at 90°C. for 8 hours. The two alkali extracts were combined and treated with enough concentrated

HCl to form a solution which was 0.5 N in HCl. The solid organic matter was allowed to flocculate and settle, and only the supernatant was used in the experiment. Twelve-gram samples of the pretreated soil were extracted with a total of 180 ml. of 0.5 N NaOH in a similar manner to provide an extract which was about twelve times more concentrated.

Different volumes of supernatant from the two extracts were added to chromatographic columns which had been prepared with 10 g. of carbon. The columns were eluted with a total volume of 300 ml. of 0.5 N HCl. After 150 ml. of the acid had passed through the columns, the elution process was interrupted for an overnight delay. The eluate was collected in 50-ml. increments and analyzed for total and inorganic phosphorus. The phosphorus content of the ashed carbon was also determined.

Results and discussion

The experimental results are listed in Table 6. The data are averages of treatments applied to duplicate columns. The values for phosphorus in the ashed carbon have been corrected for a blank. The organic phosphorus content of the two NaOH extracts was determined by the method of difference. The extract from 1 g. of soil with 180 ml. of 0.5 N NaOH contained 19.0 µg. of organic phosphorus per 10 ml. The extract from 12 g. of soil with 180 ml. of NaOH contained 208 µg. of

Table 6. Organic and inorganic phosphorus in increments of eluate, and phosphorus sorbed on carbon from chromatographic columns treated with different volumes of NaOH extracts of soil

Volume of extract added to columns, ml.	Kind of phosphorus	Phosphorus values obtained by method of difference, µg. Added to columns	Present in indicated portion of eluate: 1st 50 ml.	2nd 50 ml.	3rd 50 ml.	4th 50 ml.	5th 50 ml.	6th 50 ml.	Phosphorus recovered in ashed carbon: µg.	Percent of added organic phosphorus
Extract from 1 g. of soil with 180 ml. of 0.5 N NaOH										
5	Organic	9.5	2.0	0.5	-1.3[a]	0.8	0.0	-0.2	8.8	93
	Inorganic	1.6	1.7	0.3	5.4	0.2	0.0	0.2		
20	Organic	37.8	2.0	0.3	-0.3	0.8	-0.5	-0.2	36.0	95
	Inorganic	6.5	6.5	0.5	0.9	0.1	4.9	0.2		
60	Organic	123	1.0	0.4	0.2	0.6	0.1	-0.1	106	86
	Inorganic	19.4	15.2	0.3	0.5	6.1	0.3	0.1		
Extract from 12 g. of soil with 180 ml. of 0.5 N NaOH										
5	Organic	104	7.6	0.6	1.9	0.7	0.7	-0.3	97.8	94
	Inorganic	17.0	14.7	0.5	0.3	0.4	0.0	8.0		
20	Organic	418	35.8	1.8	0.7	1.3	2.8	0.9	379	91
	Inorganic	68.0	57.2	1.2	1.6	0.7	3.2	0.7		
60	Organic	1254	21.9	3.5	1.8	9.9	6.1	0.3	1092	87
	Inorganic	204	167	0.9	2.8	2.1	0.6	2.9		

[a]Negative entries result from analytical values for inorganic phosphorus that exceed the corresponding analytical values for total phosphorus.

organic phosphorus per 10 ml.

From 86 to 95% of the added organic phosphorus was sorbed on the 10 g. of carbon. A higher percentage of organic phosphorus was sorbed on carbon from the smaller aliquots than from the larger aliquots. For a given size of aliquot the carbon sorbed approximately the same proportion of organic phosphorus from the concentrated alkali soil extract as from the more dilute extract. Up to 21% of the added organic phosphorus was recovered in the first 50-ml. increment of eluate. Some leakage of organic phosphorus from the columns which had received the larger volumes of concentrated extract occurred almost continuously during the elution process.

The recovery of significant quantities of organic phosphorus in the first increment of eluate demonstrated that either part of the organic phosphorus in the NaOH extract was not sorbed on the carbon or was sorbed so weakly that it was readily leached from the column by the first 50 ml. of 0.5 N HCl. Because a considerable volume of 0.5 N HCl is needed to elute the inorganic phosphorus, it is evident that the experimental conditions employed here do not provide a quantitative separation of organic and inorganic phosphorus.

Comparison of NaOH Extracts and NH_4OH Extracts Obtained with Different Extraction Procedures from an Alkaline Soil in Columns Containing 10 g. of Carbon

Introduction

The chromatographic separation of organic phosphorus from NaOH extracts, investigated in the previous experiment, was not satisfactory. Significant amounts of organic phosphorus were leached from the carbon column by the first portion of HCl eluant.

The sorptive tendencies of carbon for organic phosphorus compounds may rely in part on certain inherent, unalterable properties of the phosphorus compounds, in which case neither the extracting reagents nor the extraction procedure would affect the success of the chromatographic separation. On the other hand, the sorptive behavior of carbon for organic phosphorus may be a function of certain properties of the phosphorus compounds which are readily altered by the type of extractant or by the extraction procedure. NaOH is a much stronger alkali than NH_4OH (another reagent that has been used extensively for extracting organic phosphorus from soil) and is known to cause greater hydrolysis of organic phosphorus to inorganic form than does NH_4OH. NaOH probably causes greater splitting of other linkages also, so that the individual units carrying phosphorus in organic form will tend to be smaller in NaOH extracts than in NH_4OH extracts and less

readily adsorbed by carbon. On the possibility that the separation of organic and inorganic phosphorus might be more nearly quantitative where the extraction is made with NH_4OH than with NaOH, an experiment was conducted to compare the two extractants. The experiment was designed to provide at the same time a test of the hypothesis about the effect of splitting of the organic phosphorus compounds on their sorption by carbon.

Procedure

Ten-gram samples of Harpster silty clay loam F-2868, previously treated with N HCl, were extracted with 140 ml. of 0.5 N NaOH at room temperature for 1 hour. The soil residue and the extract were separated by centrifugation and decantation. A 50-ml. portion of the alkali extract was acidified with concentrated HCl and adjusted to a final volume of 100 ml. of 0.5 N HCl. The suspended, solid, organic material was allowed to settle, and the supernatant was removed and labeled "cold-NaOH extract". A second 50-ml. portion of the alkali extract was heated at 90°C. for 8 hours, then acidified with HCl and diluted to a final volume of 100 ml. of 0.5 N HCl. The supernatant was removed and labeled "heated cold-NaOH extract".

Following the initial NaOH extraction which was described above, the soil residue was extracted with a second 140-ml.

portion of 0.5 N NaOH at 90°C. for 8 hours. The second alkali extract was removed from the residue by centrifugation and decantation. A 50-ml. portion was acidified with HCl to form 100 ml. of 0.5 N HCl. After precipitation of the solid material, the supernatant was removed and labeled "hot-NaOH extract".

Other samples of the pretreated soil were simultaneously extracted with 0.5 N NH_4OH by an identical procedure, and the supernatants were labeled "cold-NH_4OH extract", "heated cold-NH_4OH extract", and "hot-NH_4OH extract".

Twenty-milliliter aliquots of the various alkali extracts were added to duplicate chromatographic columns which had been prepared with 10 g. of carbon. The columns were eluted with a total of 300 ml. of 0.5 N HCl. The elution process was interrupted for an overnight delay following the elution of the first 150 ml. of eluate. The eluate was collected in 50-ml. increments, and the individual portions were analyzed for inorganic and organic phosphorus. The phosphorus content of the ashed carbon was also determined.

Results and discussion

The experimental treatments and results are given in Table 7. The values of phosphorus recovered in the ashed carbon have been corrected for the blank. From 89 to 97% of the organic phosphorus of the added NaOH extracts was re-

Table 7. Organic and inorganic phosphorus in increments of eluate, and phosphorus sorbed on carbon from chromatographic columns treated with various alkali extracts of an alkaline soil, Harpster silty clay loam

Kind of extract added to columns	Kind of phosphorus	Phosphorus values obtained by method of difference, μg.							Phosphorus recovered in ashed carbon	
		Added to columns	Present in indicated portion of eluate							
			1st 50 ml.	2nd 50 ml.	3rd 50 ml.	4th 50 ml.	5th 50 ml.	6th 50 ml.	μg.	Percent of added organic phosphorus
Cold-NaOH	Organic	228	0.3	0.2	0.7	-0.2	-0.4	-0.2	222	97
	Inorganic	23.0	19.3	0.9	0.3	0.9	0.4	0.2		
Heated Cold-NaOH	Organic	215	4.5	0.6	1.1	-0.3	2.5	-0.3	203	94
	Inorganic	56.7	53.2	1.2	0.4	1.1	0.3	0.3		
Hot-NaOH	Organic	116	3.2	-0.4	-0.3	0.3	3.3	0.0	104	89
	Inorganic	39.2	36.1	1.1	0.3	0.7	0.2	0.0		
Cold-NH_4OH	Organic	133	-0.4	0.6	0.5	0.5	0.0	0.0	133	100
	Inorganic	12.3	10.1	0.2	0.3	0.4	0.0	0.0		
Heated Cold-NH_4OH	Organic	153	-0.4	0.1	0.0	0.5	0.3	1.1	153	100
	Inorganic	21.9	20.7	0.4	0.0	0.5	0.2	0.0		
Hot-NH_4OH	Organic	53.1	-0.1	0.8	1.6	0.5	1.0	0.8	52.7	99
	Inorganic	20.2	19.8	0.4	0.3	0.6	0.2	0.2		

covered in the ashed carbon. With increasing severity of the extraction treatment, a decreasing proportion of the organic phosphorus was recovered on the carbon. As was true in the previous experiments with NaOH extracts, some of the organic phosphorus was eluted from the columns in the first increment of 0.5 N HCl eluate.

The recovery in the ashed carbon of the organic phosphorus from the NH_4OH extracts was almost quantitative, regardless of the severity of the extraction treatment. In no case was organic phsophorus detected in the first increment of eluate from the columns which had received the NH_4OH extracts.

These results show that there was a definite difference between the two extractants. With NH_4OH as extractant, the quantity of phosphorus retained by the carbon was essentially equal to the quantity added to the columns, according to analyses by the method of difference. Quantities of phosphorus sorbed from NaOH extracts were invariably smaller than those added, according to analyses by the method of difference. The hypothesis about a difference in sorption of organic phosphorus removed from soil by the two extractants thus is verified. Because NaOH extracts more organic phosphorus than NH_4OH, an alternative explanation for the lesser retention of the phosphorus of NaOH extracts by carbon is that the less-well-retained phosphorus found in NaOH extracts was not extracted from the soil by the NH_4OH. This possibility is con-

sistent with but does not account for the effect of heating in decreasing the retention of phosphorus of the cold-NaOH extract.

Furthermore, the results suggest that the method of difference and the method of sorption are measuring the same thing, organic phosphorus, and that the low results obtained by the method of sorption in previous experiments were a consequence of leakage of some of the organic phosphorus into the eluate before analysis of the carbon. This view of course is supported by analyses by the method of difference indicating the presence of organic phosphorus in the eluate from columns treated with NaOH extracts.

Comparison of NaOH Extracts and NH_4OH Extracts Obtained with Different Extraction Procedures from an Acid Soil in Columns Containing 10 g. of Carbon

Introduction

The purpose of this experiment was to learn if the results obtained in the previous experiment could be duplicated using extracts from a different soil. In the previous experiment the chromatographic separation of the organic phosphorus from NH_4OH extracts of an alkaline soil of pH 7.5 were much more complete than from NaOH extracts of the same soil. The soil used in this experiment was an acid soil of pH 6.4.

Procedure

The extraction procedure with 0.5 N NaOH and with 0.5 N NH_4OH, the chromatographic separation, and the analyses of the eluates and of the ashed carbon were performed on samples of Nicollet loam precisely as described in the previous experiment.

Results and discussion

The experimental results are presented in Table 8. The values of the phosphorus content of the ashed carbon have been corrected for a blank. From 93 to 98% of the organic phosphorus content of the NaOH extracts of the acid soil was recovered in the ashed carbon. Maximum recovery from the three NaOH extracts was on carbon from columns treated with the cold-NaOH extract. All the organic phosphorus was recovered in the ashed carbon from columns which had received aliquots of the NH_4OH extracts. Again, no organic phosphorus was detected in the first increment of eluate from any of the columns which had received the NH_4OH extracts.

The results of this experiment with an acid soil were in agreement with the results of the previous experiment with an alkaline soil. The organic phosphorus extracted from soil samples by NH_4OH was almost completely sorbed by the chromatographic carbon columns. The severity of the heat treatments applied to the NH_4OH extracts during or after extraction did

Table 8. Organic and inorganic phosphorus in increments of eluate, and phosphorus sorbed on carbon from chromatographic columns treated with various alkali extracts of an acid soil, Nicollet loam

Kind of extract added to columns	Kind of phosphorus	Phosphorus values obtained by method of difference, μg. Added to columns	Present in indicated portion of eluate 1st 50 ml.	2nd 50 ml.	3rd 50 ml.	4th 50 ml.	5th 50 ml.	6th 50 ml.	Phosphorus recovered in ashed carbon μg.	Percent of added organic phosphorus
Cold-NaOH	Organic	142	1.5	0.2	1.0	-0.1	0.0	0.0	139	98
	Inorganic	35.5	34.4	0.5	0.0	0.1	0.0	0.0		
Heated Cold-NaOH	Organic	137	4.0	0.1	-0.1	0.1	0.0	0.0	128	93
	Inorganic	62.7	60.8	1.0	0.1	0.2	0.0	0.0		
Hot-NaOH	Organic	48.0	-1.3	-0.1	0.3	-0.5	0.1	-0.2	45.0	94
	Inorganic	23.9	23.8	0.5	0.0	0.5	0.0	0.2		
Cold-NH_4OH	Organic	49.0	0.0	1.9	2.1	0.8	0.4	0.0	50.3	103
	Inorganic	13.7	13.8	0.3	0.0	0.0	0.0	0.0		
Heated Cold-NH_4OH	Organic	41.5	-1.5	2.1	1.5	0.8	0.0	0.0	43.9	106
	Inorganic	20.5	20.5	0.3	0.2	0.0	0.0	0.0		
Hot-NH_4OH	Organic	42.5	-1.8	1.9	2.2	0.1	0.4	0.4	42.8	101
	Inorganic	25.2	24.5	0.5	0.1	0.1	0.0	0.0		

not affect the chromatographic separation. The separation of organic phosphorus from NaOH extracts of the acid as well as the alkaline soils was not satisfactory. Increasing severity of the heat treatments applied to the NaOH extracts of both the acid and the alkaline soils resulted in the decreasing sorption and recovery of the organic phosphorus in the carbon columns.

In contrast to the preceding experiment, the phosphorus retained from NH_4OH extracts by the carbon columns was a little greater than the phosphorus added, according to the method of difference. If the organic phosphorus found by the method of difference in the eluates is added to the phosphorus retained by the carbon, the total is definitely greater than the quantities added, according to analyses by the method of difference. Hence, in contrast to the preceding experiment the results of this experiment suggest that the method of difference and the method of sorption by carbon may not be measuring the same thing in all soils. Alternatively, some hitherto undetected interference may be affecting results by one or both methods in certain soils.

On the basis of the experimental results with NaOH extracts of soils it was decided to abandon the use of NaOH as the alkali extractant of soil organic phosphorus. The extraction method of Mehta *et al.* (1954) which employed concentrated HCl and 0.5 N NaOH as extractants was not used in any subse-

quent experiment.

Comparison of NH_4OH Extracts Obtained with Different Extraction Procedures from an Acid and an Alkaline Soil in Columns Containing 10 g. of Carbon

Introduction

In a previous experiment using extracts of an alkaline soil the chromatographic separation of organic phosphorus from NH_4OH extracts was much more satisfactory than from NaOH extracts. Similar results were obtained in the comparison of alkali extracts from an acid soil. In this experiment only NH_4OH extracts, obtained with different extraction procedures from both the alkaline and the acid soil samples, were studied chromatographically. This experiment is a repetition of the principal features of the parts of the preceding two experiments involving NH_4OH extracts, and it was conducted to verify the results of those experiments.

Procedure

The extraction procedure with 0.5 N NH_4OH and the chromatographic separation were performed simultaneously on samples of an acid soil and an alkaline soil as conducted in the preceding two experiments. The columns were eluted with a total volume of 300 ml. of 0.5 N HCl. Only the first 50-ml. increment of eluate was collected and analyzed for inorganic and organic phosphorus. The phosphorus content in the ashed

carbon was determined.

Results and discussion

The experimental data are given in Table 9. The values for phosphorus in the ashed carbon, averages of duplicate columns, have been corrected for a blank. In no case was any appreciable amount of organic phosphorus detected in the first 50-ml. increment of eluate. Although the quantities of phosphorus retained by the carbon in this experiment generally agreed closely with the quantities of organic phosphorus added (as determined by the method of difference), the results of this experiment were not quite like those of the preceding two. The quantities of phosphorus retained by the carbon from extracts of Nicollet loam were a little lower than the quantities of organic phosphorus added to the columns (according to analyses by the method of difference) in this experiment, but they were a little higher in the preceding experiment. The value of 91% recovery of the organic phosphorus of the hot-NH_4OH extract of the Nicollet loam is the lowest percentage recovery obtained in the three experiments. It compares with the figure of 101% recovery obtained in the preceding experiments. As a whole, these experiments provide no definite basis for the supposition that the method of difference and the method of sorption by carbon do not yield essentially the same answer where NH_4OH extracts are concerned.

Table 9. Organic and inorganic phosphorus in the first increment of eluate, and phosphorus sorbed on carbon from chromatographic columns treated with various NH_4OH extracts of an acid and an alkaline soil

Kind of soil	Kind of extract added to columns	Kind of phosphorus	Phosphorus values obtained by method of difference, µg. Added to columns	Present in 1st 50 ml. of eluate	Phosphorus recovered in ashed carbon µg.	Percent of added organic phosphorus
Harpster silty clay loam, pH 7.5	Cold-NH_4OH	Organic	214	-0.9	217	101
		Inorganic	28.2	23.7		
	Heated cold-NH_4OH	Organic	237	-0.7	243	102
		Inorganic	50.2	44.7		
	Hot-NH_4OH	Organic	60.6	-0.4	59.0	97
		Inorganic	23.9	23.2		
Nicollet loam, pH 6.4	Cold-NH_4OH	Organic	69.4	0.5	67.9	98
		Inorganic	17.2	16.8		
	Heated cold-NH_4OH	Organic	67.2	-1.0	65.9	98
		Inorganic	27.1	26.4		
	Hot-NH_4OH	Organic	33.5	-1.2	30.6	91
		Inorganic	23.2	22.9		

It should be noted that this experiment provides a critical test of recovery of organic phosphorus of NH_4OH extracts in the carbon columns. The hot-NH_4OH extract contains only a portion of the organic phosphorus, where the hot extraction is preceded by a cold-NH_4OH extraction, and thus the overall recovery would exceed that obtained with the hot-NH_4OH extract. Moreover, in all the extracts, work was done only with the portion of organic phosphorus that remained soluble in the 0.5 N HCl solution. The portion of the organic phosphorus present in the organic matter that flocculated upon acidification would be retained by the carbon by simple mechanical filtration, and this would tend to raise the overall percentage recovery if the recovery of one or more of the soluble fractions were low.

In general, the results of the last three experiments indicate that the development of an extraction procedure using NH_4OH as the alkali extractant is warranted.

The Flocculating Effect of NH_4Cl on Organic Phosphorus in NH_4OH Extracts of Soils

Introduction

With the selection of NH_4OH as the alkali extractant of soil organic phosphorus in preference to NaOH, the extraction procedure of Mehta et al. (1954) could no longer be used to provide the soil extracts for the development of the chromato-

graphic columns. Accordingly, published extraction procedures which employ NH_4OH were considered from the aspect of their application to the chromatographic carbon columns.

One method of extracting soil organic phosphorus using NH_4OH as the alkali extractant is that of Pearson (1940), as modified by Thompson (1950). In the modified version, soil samples are extracted with N HCl and then with 0.5 N NH_4OH at 90°C. for 16 to 18 hours. The two extracts, the acid and the alkali, are not combined but kept separate. Organic phosphorus is determined in the two extracts by measuring inorganic phosphorus in combined aliquots before and after ignition to convert all the phosphorus to inorganic form. The difference between total and inorganic phosphorus is taken to represent organic phosphorus. Before removing aliquots of the alkali extract for phosphorus determination, 5 g. of NH_4Cl are added to the 400 ml. of 0.5 N NH_4OH extract to facilitate the precipitation of suspended solids.

Because the flocculating effect of NH_4Cl was not clearly understood and because it was not certain how the NH_4Cl might affect the chromatographic separation of organic and inorganic phosphorus, this first experiment in the investigation of the NH_4OH extraction method was conducted to measure the effect of NH_4Cl on flocculation of solids in the alkali extract.

Procedure

One-gram samples of Harpster silty clay loam F-2868, pretreated with N HCl, were extracted in 100-ml. centrifuge tubes with 50 ml. of 0.5 N NH_4OH for 1 hour at room temperature. At the end of the extraction period, 1 g. of NH_4Cl crystals was added to some of the tubes, and the ingredients were mixed. After 10 minutes all tubes were centrifuged in an International No. 2 centrifuge. The supernatants were carefully separated from the soil residues by decantation. The untreated supernatants, i.e., the supernatants which had not received NH_4Cl, were divided into two equal volumes, and to one volume was added 1 g. of NH_4Cl. The salt and supernatant were mixed and allowed to react for 10 minutes. Both volumes were centrifuged and decanted to separate the supernatant from the precipitate. One half of each of the three extracts was filtered under 2 atmospheres of N_2 pressure through a membrane filter having a pore size of 0.23 microns. Organic phosphorus was determined by the method of difference between total and inorganic phosphorus in each of the filtered and unfiltered solutions.

Results and discussion

The results of the experiment are given in Table 10. Maximum values of organic phosphorus were found in the supernatant which had not received NH_4Cl at any time in the pro-

Table 10. Organic phosphorus in filtered and unfiltered supernatants of NH_4OH extracts of soil with and without a flocculating agent, NH_4Cl

Addition of NH_4Cl	Organic phosphorus in 50 ml. of supernatnat, µg.	
	Filtered	Unfiltered
None	197	202
To soil and extract before separation	92	105
To supernatant after separation from soil residue	160	192

cedure. Filtering the untreated supernatant through the membrane filter reduced the organic phosphorus value by less than 3%, which indicates that a relatively clean separation of supernatant and soil residue can be achieved by centrifugation and decantation without the addition of a flocculating agent.

The lowest concentration of organic phosphorus was found in the supernatant which had been treated with NH_4Cl prior to the separation of the supernatant and soil residue. The addition of the flocculating agent to the soil-extractant system resulted in the loss of about one half of the organic phosphorus initially extracted by the 0.5 N NH_4OH. Filtering the treated supernatant through the membrane filter reduced the organic phosphorus content about 10% compared to the unfiltered portion of the same extract.

The results of this experiment indicate that the phosphorus-bearing organic matter of NH_4OH extracts flocculates to some extent by itself in the presence of NH_4Cl and that its flocculation is greatly enhanced by the presence of flocculating mineral particles. Probably the organic matter is present in part in the floccules of mineral particles. This behavior discloses an important limitation of methods using NH_4Cl in NH_4OH extracts for estimating the total organic phosphorus content of soil. To judge from the results with the single soil employed in this experiment, a significant part of the phosphorus that is extracted by NH_4OH is flocculated upon addition of NH_4Cl and is removed from the extract before the analysis is carried out.

Extraction of Soil Organic Phosphorus Using 1 N HCl and 0.5 N NH_4OH

Several preliminary experiments were conducted to aid in developing a method for extracting organic phosphorus from soil using NH_4OH without NH_4Cl as a flocculating agent. The general objective was to obtain an extract suitable for use with the carbon columns and, at the same time, to obtain good extraction of organic phosphorus without appreciable hydrolysis.

It was inferred from the work of Pearson that a hot extraction with NH_4OH was necessary to obtain a large yield of soluble organic phosphorus. The first experiment was

conducted to show the effect of sustained heating at 90°C. on the normality of a dilute solution of NH_4OH. Fifty milliliters of 0.5 N NH_4OH were placed in a 125-ml. Erlenmeyer flask. The flask was stoppered with a Bunsen valve and then heated at 90°C. for 40 hours. The flask and contents were cooled, and the alkali was titrated with a standard acid. The normality of the NH_4OH was found to be 0.48. Hence, little volatilization of ammonia occurred under the conditions studied in this experiment.

The effect of heating NH_4OH extracts on the hydrolysis of organic phosphorus was investigated next (Sekhon[1]). One-gram samples of Harpster silty clay loam F-2868, a soil which was known to have a high content of organic phosphorus, were extracted with 20 ml. of N HCl with heating to 70°C. in 10 minutes. Fifty ml. of N HCl were added to the mixture, and the solution was allowed to stand at room temperature for 1 hour. The acid extract was removed by centrifugation and decantation. Seventy ml. of 0.5 N NH_4OH were added to the soil residue and mixed. The solution was allowed to stand for 1 hour at room temperature. The NH_4OH extract was removed from the soil residue by centrifugation and decantation. The alkali extract was filtered through a membrane filter having a 0.23-micron pore size. Aliquots of the NH_4OH extract were placed into two series of matched colorimeter tubes. One series of tubes which had been loosely stoppered was placed in

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[1]G. S. Sekhon, Ames, Iowa. Hydrolysis of organic phosphorus. Private communication. 1962.

an oven and heated at 90°C. After periods of 4, 8, and 16 hours, pairs of heated and room-temperature tubes were analyzed for organic phosphorus by the method of difference. The results of the organic phosphorus determinations showed that no hydrolysis occurred in the NH_4OH extracts which were kept at room temperature for up to 16 hours. In contrast, hydrolysis of organic phosphorus did occur in all extracts heated to 90°C. Four per cent of the organic phosphorus hydrolyzed in 4 hours, 6.5% in 8 hours, and 10.7% in 16 hours. The results of the experiment demonstrated that some organic phosphorus is lost by hydrolysis when NH_4OH extracts of soils taken at room temperature are heated. From these results, one may infer that a single extraction with hot NH_4OH will cause some loss of organic phosphorus by hydrolysis and that at least some of this loss can be avoided by first extracting the soil with NH_4OH at a low temperature.

The effect of two 90°C. extractions with NH_4OH, following the first NH_4OH extraction at room temperature, was investigated next. One-gram samples of soil F-2868 were extracted with N HCl as described in the previous experiment on hydrolysis. The soil samples were extracted with 50 ml. of 0.5 N NH_4OH at room temperature for 1 hour. The samples were then subjected to two consecutive 60-ml. NH_4OH extractions at 90°C., each for 8 hours. The four individual extracts, one acid and three alkali, were filtered under pressure through the membrane filters. Organic phosphorus was

determined in each extract by the method of difference between total and inorganic phosphorus.

The total organic phosphorus extracted from the soil sample by the four extracts, one acid and three alkali, was 448 μg. of phosphorus. The proportion of the total organic phosphorus found in the individual extracts was as follows: 28% in the acid extract, 35% in the cold-NH_4OH extract, 30% in the first hot-NH_4OH extract, and 7% in the second hot-NH_4OH extract. On the basis of the low yield of organic phosphorus in the second hot-NH_4OH extract it was decided to dispense with the second hot alkali extraction in all subsequent extraction procedures.

The next study was concerned with the effect of duration of the 90°C. NH_4OH extraction. One-gram samples of soil F-2868 were extracted with 50 ml. of N HCl followed by an extraction with 60 ml. of 0.5 N NH_4OH at room temperature for 1 hour. The soil residues were then extracted with 50 ml. of 0.5 N NH_4OH at 90°C. Half of the samples were removed from the oven after a 4-hour extraction period; the other samples were extracted for 8 hours. The individual extracts were filtered through the membrane filters and then analyzed for organic phosphorus by the method of difference. A total of 494 μg. of organic phosphorus was extracted by the procedure which included a 4-hour NH_4OH extraction at 90°C. A total of 502 μg. of organic phosphorus was extracted by the procedure

which included an 8-hour hot-NH_4OH extraction. For convenience the 4-hour hot alkali extraction was selected.

The flocculating effect of NH_4Cl on organic phosphorus, revealed in the investigation of Pearson's method, was considered in developing a new extraction procedure with N HCl and 0.5 N NH_4OH. In the procedure as worked out thus far, the soil samples are extracted with N HCl and then with 0.5 N NH_4OH. The HCl remaining with the soil residue following centrifugation and decantation of the acid extract is neutralized with the first addition of NH_4OH, forming about 1 millimole of NH_4Cl. In a volume of 70 ml., 1 millimole of NH_4Cl would result in a solution having a salt concentration of about 0.02 molar (compared with a 0.03-molar concentration in the method of Pearson). This salt concentration may be sufficiently high to decrease the effectiveness of the first NH_4OH extraction because of formation of a buffer solution and precipitation of some of the organic phosphorus.

An attempt was made to prevent the formation of NH_4Cl as a result of the neutralization reaction by including an auxiliary extraction with water after the acid extractant was removed and before the first addition of NH_4OH. The inclusion of the water extraction in the procedure was not satisfactory. When the auxiliary water extraction was included, the subsequent NH_4OH extracts would not filter properly. Solid particles remained in suspension in the alkali extracts even

after sustained centrifugation at high speeds using a table-model Servall Superspeed centrifuge. This behavior is evidence for the importance of NH_4Cl in flocculation; nevertheless, the auxiliary water extraction was omitted from the procedure because the problem of filtering could not be resolved where the water extraction was included.

Because filtering through the membrane filters was somewhat of a problem even without including the auxiliary water extraction, the procedure was modified to make use of a Servall Superspeed centrifuge, which was more effective than the International No. 2 centrifuge used earlier to handle the larger volumes of extract. The volume of the individual extracts was reduced to 35 ml., since the Servall centrifuge tubes had a total capacity of only 50 ml. To maintain a wide soil-to-extractant ratio, the soil sample was reduced to 0.5 g. Pearson (1940) had reported that increasing amounts of soil organic phosphorus were extracted from soil samples by increasing the volume of NH_4OH extractant.

Although the importance of the initial acid extraction was recognized (Tinsley and Salam, 1961) the effect of acid strength was not examined. Instead, an acid extraction procedure much like the one used by Van Diest (1957) was employed. The soil sample was extracted with N HCl with heating to 70°C. in 10 minutes. The extract was removed by centrifugation and decantation. The sample was then extracted with

a second portion of N HCl at room temperature for 1 hour. Each of the individual acid and alkali extracts was filtered through a membrane filter having pores of 0.23-micron size (Superstrength Process Polypore, type 1120, supplied by the Gelman Instrument Co., 106 N. Main St., Chelsea, Michigan) using special pressure-filtering apparatus designed by Peaslee (1960). One filter was used for all extracts obtained from a given soil sample. The filtrates were collected in a single 125-ml. Erlenmeyer flask calibrated at 140 ml. After the fourth extract had been filtered into the flask, the combined extracts were treated with concentrated HCl so that upon dilution to volume with water, the flask contained 140 ml. of 0.5 N HCl (and about 0.25 N NH_4Cl). A stepwise description of the entire extraction process is given below.

A one-half-gram sample of soil (less than 60-mesh) is weighed into a 50-ml. polypropylene centrifuge tube. Ten milliliters of 1 N HCl are added, and the mixture is stirred. The tube is placed in a water bath and heated to 70°C. in 10 minutes. The mixture is stirred and cooled. The soil residue is precipitated by centrifugation at top speed for 15 minutes in a Servall Superspeed centrifuge. The acid extract is decanted into the filter apparatus and filtered under 2 atmospheres of pressure into a 125-ml. Erlenmeyer flask calibrated at 140 ml. Thirty-five milliliters of 1 N HCl are added to the residue in the tube. The mixture is stirred,

allowed to stand 1 hour at room temperature, and restirred. The contents are separated by centrifuging at top speed for 20 minutes, and the acid supernatant is decanted into the filter apparatus and filtered into the Erlenmeyer flask.

Thirty-five milliliters of 0.5 N NH_4OH are added to the soil residue; the mixture is stirred and allowed to stand for 1 hour at room temperature. The soil residue is precipitated by centrifugation at top speed for 25 minutes. The alkali supernatant is removed by decantation and filtered into the Erlenmeyer flask. Thirty-five milliliters of 0.5 N NH_4OH are added to the soil residue, and the contents are stirred. The tube is stoppered with a No. 6 rubber stopper equipped with a Bunsen valve. The tube is heated at 90°C. for 4 hours. The mixture is cooled and stirred. The contents are separated by centrifugation at top speed for 30 minutes, and the alkali supernatant is decanted into the filter apparatus and filtered into the Erlenmeyer flask.

Five milliliters of concentrated HCl are mixed with the solution in the flask. The solution is then made to a volume of 140 ml. and thoroughly mixed.

To determine the organic phosphorus in the extract by the method of difference, an aliquot of the extract, including suspended organic matter, is placed in a thoroughly weathered 50-ml. beaker. A five-milliliter aliquot of 6 N NH_4OH and a 1-ml. aliquot of a 10% solution of $Mg(NO_3)_2$

are added to the beaker. The contents are evaporated to dryness on a steam plate. The beaker is then placed in a muffle furnace and heated to 200°C. for 1 hour, and then heated to 500°C. for 2 hours. The beaker is removed and cooled. Twenty milliliters of N HCl are added to the beaker, and the beaker is heated for 10 minutes on a steam plate. After the solution has cooled, the solution is transferred to a 50-ml. volumetric flask. The contents are diluted to volume with water and thoroughly mixed. The phosphorus content of the solution is determined by the method of Dickman and Bray (1940) as modified by Legg and Black (1955). This measurement gives the total phosphorus in the extract. The suspended organic matter in the extract is allowed to settle. An aliquot of the supernatant is placed directly into a colorimeter tube for inorganic phosphorus determination by the method mentioned above. The difference between the contents of total phosphorus and inorganic phosphorus in the extract provides a measure of the content of organic phosphorus.

The determination of organic phosphorus by the method of difference described above is more convenient than the method of difference required for an extraction procedure which employs NaOH as the alkali extractant, such as the procedure of Mehta *et al*. (1954). The presence of sodium ions in the extract solution prohibits the employment of an ignition type of oxidation process unless the extract is ignited

in platinum or silica vessels. Ignition of beakers containing sodium salts results in high and variable blank values because of the excessive attack of the sodium salts on the glass. Therefore, extracts which contain sodium are oxidized by a wet digestion process such as digestion with perchloric acid. Such digestions must be watched to avoid complete evaporation of the perchloric acid. On the other hand, soil extracts obtained with NH_4OH as the alkali extractant can be ignited in Pyrex glassware in a muffle furnace unattended.

Chromatographic Determination of Organic Phosphorus in Soil Extracts Obtained by the New Extraction Procedure

Introduction

This experiment was conducted to investigate the performance of the combined extracts obtained by the new extraction procedure when the acidified mixture is applied to carbon columns. At the same time, the experiment included a test of the effect of NH_4Cl on the chromatographic separation and a preliminary test of validity of the chromatographic separation.

Procedure

One-half-gram samples of Harpster silty clay loam F-2868, were extracted by the new method described in the previous section, and 10- and 20-ml. aliquots of the extract were taken

for chromatographic separation in duplicate columns prepared with 10 g. of carbon. The columns were eluted with 300 ml. of 0.5 N HCl or 300 ml. of a solution which was 0.5 N in HCl and 0.25 N in NH_4Cl. The phosphorus content of the ashed carbon was determined.

Results and discussion

The experimental data, corrected for a blank, are given in Table 11. The organic phosphorus content of the soil

Table 11. Phosphorus sorbed on 10 g. of carbon in columns treated with aliquots of soil extract obtained by the new extraction procedure

Volume of soil extract added to columns, ml.	Kind of eluant	Phosphorus sorbed on carbon, μg.
10	0.5 N HCl	24.8
20	0.5 N HCl	50.4
20	0.5 N HCl plus 0.25 N NH_4Cl	49.9

extract used in the experiment was 50.1 μg. of phosphorus per 20 ml., as determined by the method of difference; the organic phosphorus value obtained by the chromatographic method was essentially the same. Doubling the volume of extract added to the columns resulted in doubling the amount of phosphorus recovered in the carbon. An effect of 0.25 N NH_4Cl in the

0.5 N HCl eluate was not evident.

The experimental results provide evidence on the validity of the chromatographic determination of organic phosphorus in two ways. First, a quantitative method of analysis must show recovery of the substance in question in proportion to the amount of sample analyzed. This requirement was fulfilled in this experiment. Doubling the amount of extract added to the columns resulted in doubling the quantity of phosphorus recovered in the ashed carbon. Second, although no proof can be offered concerning the accuracy of the value of organic phosphorus, the value of organic phosphorus determined chromatographically was the same as the value determined by the method of difference. Coincidence of results obtained by two independent methods provides strong verification of the accuracy of the methods.

Effect of Different Amounts of Carbon on the Chromatographic Determination of Organic Phosphorus in Soil Extracts

Introduction

The use of 10 g. of carbon in the columns was initiated in previous work in attempts to obtain a more complete recovery of the organic phosphorus from NaOH extracts. To reduce the phosphorus content of the blank, a smaller amount of carbon would be preferable, provided the smaller amount were efficient in sorbing the organic phosphorus from the soil

extract. With the change from NaOH to NH_4OH as the alkali extractant, the larger amount of carbon, 10 g., might not be required for accurate determinations. This experiment was designed to compare the effect of different amounts of carbon on the sorption of organic phosphorus from soil extracts.

Procedure

Samples of Harpster silty clay loam F-2868 were extracted by the new extraction procedure, and 25-ml. aliquots of the soil extract were added to duplicate chromatographic columns which contained 4, 6, 8, and 10 g. of carbon. The adsorbent bed formed by 4 g. of carbon was about 5 cm. in height; the bed formed by 10 g. of carbon was about 14 cm. in height. All columns were eluted with 300 ml. of 0.5 N HCl.

Results and discussion

The results of the experiment are presented in Table 12. The data have been corrected for blanks which were calculated by interpolation between two 4-g. carbon blanks and two 10-g. carbon blanks. The trend was for increasing sorption of phosphorus with increasing amounts of carbon in the columns.

The results can be interpreted in different ways. If the phosphorus content of the ashed carbon, after correcting for the blank, represents sorbed organic phosphorus, then the increase in phosphorus sorption with the increase in amount

Table 12. Phosphorus sorbed from soil extract by columns containing different amounts of carbon

Amount of carbon in column, g.	Phosphorus sorbed on carbon from 25 ml. of soil extract, μg.
4	68.4
6	68.2
8	68.9
10	69.7

of carbon in the column would indicate that some organic phosphorus was lost from the columns containing the smaller amounts of carbon. Hence, the larger amounts of carbon were providing the more reliable organic phosphorus determination.

The organic phosphorus content of the soil extract, determined by the method of difference, was 68.0 μg. of phosphorus per 25 ml. All the organic phosphorus values determined chromatographically were higher than the value calculated by difference. Although the method of difference has been used throughout this study to provide analytical values for comparison with the chromatographic method, it is not known if the method of difference is suitable as a standard. The method of difference has been used here because it is a traditional method of analysis and because no evidence is available to indicate that the results it provides are not

accurate. Several workers have tested the internal consistency of the method of difference and found it to be satisfactory.

Another possible explanation for the results found in this experiment is that increasing amounts of carbon in the columns sorbed increasing amounts of inorganic phosphorus from the soil extract. Previous investigations on the elution of inorganic phosphorus from carbon columns were concerned with columns which contained 4 g. of carbon. Larger amounts of carbon in the columns were not employed in the elution studies.

Effect of Different Amounts of Carbon in the Chromatographic Columns on the Retention of Inorganic Phosphorus

Introduction

All the previous work on the retention of inorganic phosphorus in chromatographic columns had been with columns containing 4 g. of carbon. None of the work had been with 10-g. carbon columns. An investigation of the retention of inorganic phosphorus in columns containing larger amounts of carbon was necessary to evaluate more critically the results of the previous experiment.

Procedure

Two inorganic phosphorus solutions were prepared with KH_2PO_4 in 0.5 N HCl. The concentrations were 10^4 and 10^6 μg. of phosphorus per 25 ml. Twenty-five-milliliter aliquots of the solutions were added to chromatographic columns which had been prepared with 4, 6, 8, and 10 g. of carbon. The columns were eluted with 300 or 500 ml. of 0.5 N HCl. The treatments were not replicated.

Results and discussion

The data, listed in Table 13, show that some inorganic

Table 13. Inorganic phosphorus retained by columns containing different amounts of carbon

Carbon in columns, g.	Volume of 0.5 N HCl eluant, ml.	Phosphorus retained on carbon from indicated inorganic phosphorus solutions, μg.	
		10^4 μg. of phosphorus added per column	10^6 μg. of phosphorus added per column
4	300	2.4	28.1
6	300	3.7	41.7
8	300	3.7	53.2
10	300	5.8	64.6
4	500	1.8	16.0
10	500	4.6	40.5

phosphorus was retained by the carbon in every column. Increasing quantities of inorganic phosphorus were retained on the carbon with increasing amounts of carbon in the columns. The elution with the larger volume of 0.5 N HCl reduced the retention of inorganic phosphorus on the carbon.

The results of the experiment emphasize the need for the inclusion of adequate controls or blank columns in every experiment. The advantage of using a larger volume of eluate is also indicated.

Effect of 4 and 10 g. of Carbon in the Chromatographic Columns on the Determination of Organic Phosphorus in Soil Extracts with and without Added Inorganic Phosphorus

Introduction

In a previous experiment the results of the chromatographic determination of organic phosphorus in soil extracts varied somewhat with the amount of carbon in the chromatographic column. A subsequent experiment demonstrated that larger amounts of inorganic phosphorus were retained in columns prepared with larger amounts of carbon. Thus, the effect of different amounts of carbon in the columns on the determination of organic phosphorus may be confounded by the retention of inorganic phosphorus. The purpose of this experiment was to clarify the issue by adding soil extract with and without added inorganic phosphorus to 4-g. carbon columns

and to 10-g. carbon columns.

Procedure

Twenty-five-milliliter aliquots of supernatant from extracts of Webster silty clay loam, obtained by the new extraction procedure, with and without 25-ml. aliquots of an inorganic phosphorus solution which contained 10^4 μg. of phosphorus per aliquot, were added to duplicate 4- and 10-g. carbon columns. The columns were eluted with 300 or 500 ml. of 0.5 N HCl. The phosphorus content of the ashed carbon was determined.

Results and discussion

The experimental results are presented in Table 14. The values for phosphorus content in the ashed carbon have been corrected for a blank. Twenty-five milliliters of supernatant contained 10.2 μg. of organic phosphorus, as determined by the method of difference.

Where the columns received soil extract alone, the amount of phosphorus retained did not decrease upon increasing the volume of eluant from 300 to 500 ml. These results indicate that no significant quantity of inorganic phosphorus was retained by the carbon because previous work has shown that retention of inorganic phosphorus decreases with increasing volume of eluant. The sums of the phosphorus retained from

Table 14. Effect of 4 and 10 g. of carbon on the chromatographic determination of organic phosphorus in soil extracts with and without added inorganic phosphorus

Phosphorus added per column, µg.			Volume of 0.5 N HCl eluant, ml.	Increase in phosphorus content of carbon, µg.	
Soil extract		KH_2PO_4 solution		4 g. of carbon per column	10 g. of carbon per column
Organic	Inorganic				
10.2	86.5	0	300	13.5	14.4
10.2	86.5	0	500	13.5	15.0
0	0	10^4	300	2.6	7.3
10.2	86.5	10^4	300	15.8	21.8

the soil extract and from the inorganic source added separately were essentially equal to the amounts of phosphorus retained where the soil extract and inorganic phosphorus were added together. These results show that each component acts independently. The view therefore appears justified that to measure organic phosphorus in extracts high in inorganic phosphorus, the extracts may first be analyzed for inorganic phosphorus, and the amount found may then be added to and eluted from separate columns of carbon to provide controls to correct for inorganic phosphorus retention. Where the extracts contain little inorganic phosphorus, the control obtained by leaching the columns with the same volume of HCl used in columns receiving soil extract may be adequate.

The quantity of inorganic phosphorus added in this experiment was far greater than that added in the soil extract, so that the quantities of phosphorus retained in the inorganic phosphorus columns do not provide control values for the columns receiving soil extract. Nevertheless, to judge from the negligible retention of inorganic phosphorus in previous work where the addition was small, the organic phosphorus retained from the extract was not contaminated appreciably with inorganic phosphorus. If the foregoing views are correct, the greater retention of phosphorus from soil extract by 10-g. columns than by 4-g. columns indicates some loss of organic phosphorus from the 4-g. columns.

In this experiment the phosphorus retained by the carbon from the soil extract was much greater than the organic phosphorus found by the method of difference. With nearly nine times as much inorganic phosphorus as organic phosphorus in the extract, however, the analyses by the method of difference are not highly precise.

Effect of Different Volumes of 0.5 N HCl Eluant on the Chromatographic Determination of Organic Phosphorus in Soil Extracts

Introduction

The last three experiments have been devoted to the evaluation of the effect of different amounts of carbon in the chromatographic columns on the chromatographic determination

of organic phosphorus. The results of the last experiment suggest that 10 g. of carbon may be more effective than 4 g. Both the 4-g. and the 10-g. carbon columns provided values of organic phosphorus that were higher than the value determined by the method of difference. Therefore the immediate problem seemed to be to evaluate further the validity of the chromatographic method in comparison to the method of difference rather than to study the finer points of the chromatographic method. Hence, although the 10-g. carbon columns provided a larger organic phosphorus value in the last experiment for that particular soil extract, it was decided to use 4-g. carbon columns in the future experiments. The smaller amount of carbon would provide a smaller phosphorus content in the blank and retention of inorganic phosphorus would be a lesser problem.

The source of the phosphorus recovered in the ashed carbon has not been determined. Organic phosphorus sorbed on carbon has not been differentiated from the inorganic phosphorus retained by the carbon. Previous investigations suggested a possible difference in behavior of sorbed organic phosphorus and retained inorganic phosphorus, namely, that with increasing volume of 0.5 N HCl eluant a decreasing quantity of inorganic phosphorus was retained by the carbon. Organic phosphorus sorbed on carbon from soil extracts appeared to resist leaching. This experiment was designed

to study the effect of increasing volume of eluant on phosphorus sorbed from soil extracts.

Procedure

The supernatants of several soil extracts obtained by the new extraction procedure were combined and mixed to form a large uniform supply of extract. Twenty-five-milliliter aliquots of the extract were added to chromatographic columns prepared with 4 g. of carbon. Duplicate columns were eluted with volumes of 0.5 N HCl ranging from 50 to 500 ml. The phosphorus content of the ashed carbon was determined.

Results and discussion

The results are given in Table 15. The organic phosphorus content of the soil extract was 5.25 μg. per 25 ml. as determined by the method of difference. The organic phosphorus content was 6.89 μg. per 25 ml. as determined by the chromatographic columns. The value remained virtually unchanged with a 10-fold increase in volume of 0.5 N HCl eluant.

The results of this experiment provide additional evidence on the validity of the chromatographic method of determining organic phosphorus. In this experiment the phosphorus sorbed on carbon definitely did not behave like inorganic phosphorus toward the change in volume of eluate; hence, it appears that the sorbed phosphorus was organic and that the value obtained by the method of difference was too low.

Table 15. Effect of volume of 0.5 N HCl eluate on the quantity of phosphorus sorbed from a soil extract

Volume of 0.5 N HCl eluate, ml.	Phosphorus sorbed in duplicate 4-g. carbon columns from 25 ml. of soil extract, μg.
50	6.71 7.04
100	6.89 7.04
200	6.89 6.89
300	6.82 6.89
400	6.89 6.89
500	6.89 6.89

Tests on the Validity of Organic Phosphorus Determinations by the Method of Difference and by the Method of Chromatography

Introduction

In previous work, the results of the chromatographic determination were in apparent disagreement with the results of the method of difference for several soil extracts although no statistical tests were carried out to see if the differences were significant. As will be shown subsequently, however, the two methods do give significantly different results

in some instances. If the results are definitely different, it is certain that both cannot be correct. One may inquire, therefore, as to the validity of measurements obtained by the two methods. The purpose of this experiment was to apply some tests of validity that are suitable where no absolute standard is available.

Procedure

Samples of Harpster silty clay loam F-2868 and samples of Webster silty clay loam F-2915 were extracted by the new extraction procedure. An inorganic phosphorus solution was prepared in 0.5 N HCl which contained 30 μg. of phosphorus per 5 ml. Five-milliliter aliquots of each of the two extracts and of the inorganic phosphorus solution were placed individually and in various combinations into chromatographic columns prepared with 4 g. of carbon. A second series of 5-ml. aliquots in identical combinations was placed directly into 50-ml. beakers for ignition at 500°C. and subsequent total phosphorus determination. A third series of 5-ml. aliquots was placed directly into colorimeter tubes for inorganic phosphorus determinations.

The chromatographic columns were eluted with 300 ml. of 0.5 N HCl, and the ashed carbon was analyzed for phosphorus. All determinations in both methods of analysis were duplicated.

Results and discussion

The experimental data are given in Table 16. The results of the experiment show small exceptions to the principle of additivity with both methods. The deviations, however, are too small to constitute adequate proof of invalidity of either method. Because of the relatively small difference obtained between measurements by the two methods, and because of the small number of replicates (resulting from the relatively large number of treatments), the experimental conditions did not provide a critical test of validity. Although the experiment did not provide the critical test that was desired, the results do provide evidence that the two methods do not always lead to the same value for organic phosphorus. The experiment provides four pairs of values for organic phosphorus by the two methods, each of which represents the mean of duplicate measurements. In every instance, the values obtained by the method of difference exceed those obtained by the method of chromatography.

Determination of the Inorganic Phosphorus Retained by Carbon Columns from a P^{32}-Tagged Soil Extract

Introduction

In previous experiments, the determination of the organic phosphorus content of soil extracts by the method of chromatography had provided values that were as much as 30% higher

Table 16. Determination of organic phosphorus in test solutions by the method of difference and by the method of chromatography

Volume of solution added per column, ml.			Phosphorus content of test solution by method of difference, µg.			Phosphorus sorbed on carbon from test solution, µg.
Extract of soil F-2868	Extract of soil F-2915	Inorganic phosphorus solution	Total	Inorganic	Organic	
5	0	0	19.50	9.21	10.29	9.72
10	0	0	38.05	18.25	19.80	19.03
0	5	0	12.34	8.01	4.33	3.89
5	5	0	31.35	17.21	14.14	13.83
0	0	5	30.25	30.11	0.14	0.03
5	0	5	48.95	38.02	10.93	10.11

than the values determined by the method of difference. It was reasoned that the higher values by the method of chromatography might be caused by the retention of some inorganic phosphorus on the carbon, the argument being that retention of the inorganic phosphorus of soil extracts by the carbon columns might exceed the retention of added inorganic phosphorus. In this experiment the inorganic phosphorus of the soil extract was tagged with P^{32} as a means of determining the quantity of inorganic phosphorus retained from the soil extract by the carbon. Measurement of the quantity of inorganic phosphorus retained on the carbon would make possible a correction of the phosphorus content of the ashed carbon to obtain the sorbed organic phosphorus. The corrected value could then be used in testing the comparative validity of the two methods of organic phosphorus determination.

Procedure

Samples of Ida silt loam were extracted by the new extraction procedure, and the extracts were combined to form a large uniform supply. Three special solutions were prepared in 0.5 N HCl. A solution was prepared which contained 10 μc. of P^{32} (as carrier-free orthophosphate) and 1 μg. of inorganic phosphorus per 10 ml. A second solution was prepared to serve as a check for the P^{32} solution; it contained just 1 μg. of inorganic phosphorus per 10 ml. A third solution was prepared

which contained 40 μg. of inorganic phosphorus per 20 ml. to simulate the effect of the inorganic phosphorus in the soil extract on phosphorus retention by carbon.

Aliquots of the special solutions and of the soil extract were added in various combinations to chromatographic columns which had been prepared with 4 g. of carbon. The columns were eluted with 400 ml. of 0.5 N HCl. The carbon was ashed and analyzed for phosphorus. Two-milliliter aliquots of the solution containing the dissolved carbon ash were placed on silicone-treated aluminum planchets and evaporated to dryness. The relative activity of P^{32} on the planchet was determined by a mica-window Geiger-Müller tube and scaler. The relative activity in the ashed carbon was compared to the relative activity in an aliquot of the P^{32} solution which was added to the chromatographic columns. On the assumptions that P^{32} did not exchange with organic phosphorus compounds (Gourley, 1952) and that the chemical behavior of inorganic phosphate was not dependent on the kind of phosphorus nuclide present, the following relationship was applied to the experimental results:

$$\frac{\text{μg. of inorganic phosphorus retained on the carbon}}{\text{μg. of inorganic phosphorus added to the column}} = \frac{\text{relative activity of } P^{32} \text{ retained on the carbon}}{\text{relative activity of } P^{32} \text{ added to the column}} \qquad (1)$$

The right-hand term of the equation was determined from the relative activities of the P^{32}, and the denominator of the left-hand term was determined from the colorimetric phosphorus

analyses of the solutions added to the columns. The quantity of inorganic phosphorus retained on the carbon was then calculated. The values for phosphorus sorbed on the carbon from soil extracts were corrected for the amount of inorganic phosphorus retained on the carbon.

The organic phosphorus content of the soil extract was also determined by the method of difference. The total phosphorus and the inorganic phosphorus determinations were replicated three times.

Results and discussion

The results of the chromatographic determinations are given in Table 17. The amount of inorganic phosphorus retained on the carbon from the soil extract was 0.13 μg. per column. This quantity of inorganic phosphorus represents about 1% of the total phosphorus content of the ashed carbon and an even smaller proportion of the 30.18 μg. of inorganic phosphorus added in the aliquot of soil extract. The total amount of phosphorus retained by the carbon from the soil extract was 9.10 μg. The organic phosphorus content of the same quantity of extract, as found by the method of difference, was 6.90 μg. The quantity of inorganic phosphorus retained by the carbon from the extract thus accounts for only a small part of the difference between the two methods.

The significance of the difference between the values

Table 17. Effect of the addition of soil extract with and without P^{32} on the phosphorus recovered in chromatographic carbon columns

Ml. of solution added per column								
Soil extract	$P^{32}O_4$ solution containing 1 μg. P/ 10 ml.	PO_4 solution containing 1 μg. P/ 10 ml.	PO_4 solution containing 40 μg. P/ 20 ml.	Blank solution of 0.5 N HCl	Total phosphorus content of ashed carbon, μg.	(P^{32} counts per second in carbon)/ (P^{32} counts per second in solution added)	Inorganic phosphorus retained by carbon from equation 1, μg.	Corrected phosphorus content of ashed carbon,[a] μg.
20	0	10	0	0	13.06			12.93
20	0	10	0	0	13.29			13.16
20	0	10	0	0	13.37			13.24
0	0	10	20	0	3.92			3.92
0	0	10	20	0	4.12			4.12
0	0	10	20	0	4.27			4.27
0	10	0	0	20	4.34	0.0066	0.01	4.34
0	10	0	0	20	4.12	0.0062	0.01	4.12
0	10	0	0	20	4.12	0.0056	0.01	4.12
20	10	0	0	0	13.29	0.0044	0.13	13.16
20	10	0	0	0	13.29	0.0039	0.13	13.16
20	10	0	0	0	13.35	0.0043	0.13	13.22
0	10	0	20	0	4.12	0.0038	0.15	4.12
0	10	0	20	0	4.12	0.0042	0.17	4.12
0	10	0	20	0	4.34	0.0039	0.16	4.34
0	0	0	0	30	4.27			4.27
0	0	0	0	30	4.12			4.12
0	0	0	0	30	4.12			4.12

[a]Only the phosphorus content of ashed carbon from columns which received soil extract was corrected for inorganic phosphorus, 0.13 μg. of phosphorus per column.

for organic phosphorus was next investigated to verify that the difference between 8.97 and 6.90 is not attributable to experimental error.

Both analytical methods of organic phosphorus determination are considered to be methods of difference. Just one value of organic phosphorus was calculated from the chromatographic data by taking the difference between the averages of the phosphorus content of ashed carbon from columns which received soil extract and columns which did not receive soil extract. As shown in Table 18, the corrected organic phosphorus content of the soil extract is 8.97 μg. of phosphorus per 20 ml. when calculated in this way. The organic phosphorus content of the soil extract was found to be 6.90 μg. of phosphorus as determined by the method of difference between average total phosphorus and average inorganic phosphorus contents. The calculations are given in Table 19. To determine if the two methods of analysis provided the same value of organic phosphorus a t-test was performed using the following model (Cady[1]).

$$t = \frac{z - w}{(s_z^2 + s_w^2)^{1/2}}$$

where z is μg. of organic phosphorus determined by the method of difference between the means of total and inorganic phos-

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[1]F. B. Cady, Ames, Iowa. Statistical comparison. Private communication. 1962.

Table 18. Chromatographic determination of organic phosphorus in P^{32}-tagged soil extract (the analytical values are from the last column of Table 17)

	Phosphorus content of ashed carbon from columns treated with and without 20 ml. of soil extract, µg.		Organic phosphorus, µg.
	With extract	Without extract	
	12.93	4.27	
	13.16	4.12	
	13.24	4.12	
	13.16		
	13.16		
	13.22		
Average	13.14	4.17	8.97
Variance of means)	0.00205	0.00250	0.00455
Degrees of freedom)	5	2	7

Table 19. Determination of organic phosphorus in soil extract by method of difference

	Phosphorus content in 20 ml. of soil extract, µg.		
	Total	Inorganic	Organic
	36.37	30.20	
	37.89	30.07	
	36.98	30.27	
Average	37.08	30.18	6.90
Variance of means)	0.19503	0.00343	0.19846
Degrees of freedom)	2	2	4

phorus, S_z^2 is the variance of z (equal to the sum of the variances of the means for total and inorganic phosphorus), w is μg. of organic phosphorus determined by the method of chromatography, and S_w^2 is the variance of w (equal to the sum of the variances of the means for the corrected phosphorus content of ashed carbon from columns which received soil extract and the phosphorus content of ashed carbon from columns which did not receive soil extract).

The calculated t value was 4.600 with 11 degrees of freedom. The probability of obtaining a t value as large or larger than 4.600 with 11 degrees of freedom is less than 0.001 if the results obtained by the two methods represent random values obtained from a single population. With a probability such as this, there is little doubt that the results obtained with the two methods on this soil are different. Moreover, because the quantity of inorganic phosphorus retained by the carbon from the soil extract was so small, and because the test of significance of the difference between methods was made after correcting the chromatographic values for inorganic phosphorus retained, there is little doubt that the cause of the difference in results is something other than retention of inorganic phosphorus from the extract by the carbon.

Effect of 4 and 10 g. of Carbon in the Chromatographic Columns on the Determination of Organic Phosphorus in P^{32}-Tagged Soil Extract

Introduction

The effect of different amounts of carbon in the chromatographic columns on the determination of organic phosphorus in soil extracts was studied in a number of experiments. The superiority of the larger amounts of carbon was not established, however, even though larger amounts of carbon appeared to sorb more phosphorus from the soil extracts. It was demonstrated that the larger amounts of carbon retained a larger quantity of inorganic phosphorus from inorganic phosphorus solutions. The solutions employed contained much more inorganic phosphorus than did the soil extracts. In this experiment the inorganic phosphorus content of a soil extract was tagged with radioactive phosphorus, and the tagged extract was added to chromatographic columns which were prepared with different amounts of carbon. The ashed carbon was analyzed for total phosphorus and for radioactive phosphorus. The data were used to calculate the amount of retained inorganic phosphorus. The effect of different amounts of carbon was then determined using the corrected values of sorbed organic phosphorus.

Procedure

Eighteen chromatographic columns were prepared, nine with 4 g. of carbon and nine with 10 g. of carbon. Soil extract was obtained from Albaton silt loam using the new extraction procedure. A P^{32} solution was prepared which contained 10 μc. of P^{32} and 2 μg. of inorganic phosphorus per 20 ml. of 0.5 N HCl. The experimental treatments consisted of the following additions: 1) 20 ml. of P^{32} solution, 2) 20 ml. of soil extract, and 3) 20 ml. of P^{32} solution plus 20 ml. of soil extract. Each treatment was applied to three columns within each set of nine. All columns were eluted with 400 ml. of 0.5 N HCl. The inorganic phosphorus content of the ashed carbon was determined by the usual colorimetric method of analysis. Aliquots of the ashed carbon solution were also taken for determination of the relative activity of the P^{32}. The results of the P^{32} determinations were used to calculate the amount of inorganic phosphorus retained by the carbon. A detailed description of the calculations is given in the previous section.

The organic phosphorus content of the soil extract was also determined by the method of difference. Twelve aliquots of extract were analyzed, six for total phosphorus and six for inorganic phosphorus. Organic phosphorus was calculated from the difference between the means.

Results and discussion

The experimental treatments and the results of the chromatographic determinations are given in Table 20. All values of the phosphorus content of the ashed carbon were corrected for the calculated quantity of retained inorganic phosphorus. As was found in the previous experiment which employed P^{32}, the carbon in the chromatographic columns retained only small amounts of inorganic phosphorus. In the 4-g. carbon columns the carbon retained 0.19 μg. of inorganic phosphorus from the soil extract. In the 10-g. carbon columns the average quantity of inorganic phosphorus retained on the carbon was 0.36 μg.

An examination of the corrected data in the last column in Table 20 suggested that the divergence of the next to last value may have resulted from a gross error. Accordingly, a test was made by the method of Dixon (1953), the results of which indicated a probability of less than 0.01 that the divergent value was from the same population as the other five values obtained with application of soil extract to columns carrying 10 g. of carbon. Therefore, the datum was rejected and omitted from all further calculations.

For statistical purposes the chromatographic determination of organic phosphorus is considered to be a method of difference. Organic phosphorus is calculated from the difference between the corrected phosphorus content of ashed

Table 20. Effect of addition of soil extract with and without P^{32} on the phosphorus recovered in chromatographic columns containing 4 and 10 g. of carbon

Amount of carbon per column, g.	Ml. of solution added per column: Soil extract	Ml. of solution added per column: $P^{32}O_4$ solution containing 2 µg. P/ 20 ml.	Total phosphorus content of ashed carbon, µg.	(P^{32} counts per second in carbon)/ (P^{32} counts per second in solution added)	Inorganic phosphorus retained by carbon from equation 1, µg.	Corrected phosphorus content of ashed carbon, µg.
4	0	20	4.81	0.0054	0.01	4.80
4	0	20	4.81	0.0060	0.01	4.80
4	0	20	5.21	0.0067	0.01	5.20
4	20	0	8.95		0.19	8.76
4	20	0	8.75		0.19	8.56
4	20	0	8.14		0.19	7.95
4	20	20	8.95	0.0043	0.19	8.76
4	20	20	8.32	0.0049	0.22	8.10
4	20	20	8.14	0.0036	0.16	7.98
10	0	20	9.77	0.0159	0.03	9.74
10	0	20	9.77	0.0160	0.03	9.74
10	0	20	9.55	0.0160	0.03	9.52
10	20	0	13.62		0.36	13.26
10	20	0	13.62		0.36	13.26
10	20	0	13.83		0.36	13.47
10	20	20	13.83	0.0078	0.35	13.48
10	20	20	16.01	0.0088	0.39	15.62
10	20	20	13.83	0.0077	0.35	13.48

carbon from columns which received soil extract and the corrected phosphorus content of ashed carbon from columns which did not receive extract. In this experiment the columns which received only P^{32} solution served as the blanks. The columns which received soil extract or soil extract plus P^{32} solution served as the "with extract" columns.

The results of the organic phosphorus determinations by the 4-g. and 10-g. carbon columns and by the method of difference are given in Table 21. The corrected value of sorbed

Table 21. Determination of organic phosphorus in P^{32}-tagged soil extract by 4-g. and 10-g. carbon columns and by the method of difference

Method of analysis	Organic phosphorus per 20 ml. of soil extract, µg.	Variance of organic phosphorus	Degrees of freedom
4-g. carbon columns	3.42	0.04274	7
10-g. carbon columns	3.72	0.01933	6
Difference	2.54	0.22070	10

organic phosphorus, determined by 4-g. carbon columns, is 3.42 µg. of phosphorus per 20 ml. of soil extract. The corrected value of sorbed organic phosphorus, determined with 10-g. carbon columns, is 3.72 µg. of phosphorus per 20 ml. of extract. It is noteworthy that, in this experiment, where

a proper correction could be made for the inorganic phosphorus sorbed by the carbon from the soil extract, a difference in apparent quantity of organic phosphorus in favor of the longer columns still existed. This difference, although consistent with results of previous experiments, was not significant at the 5% level.

The organic phosphorus content of the soil extract as determined by the method of difference between total and inorganic phosphorus, was 2.54 μg. of phosphorus per 20 ml. of soil extract. The value 2.54 μg. differs from the value obtained by the carbon columns at a probability level of about 12% with the 4-g. columns and at a probability level of less than 5% with the 10-g. columns.

A Comparison of the Chromatographic Determination of Organic Phosphorus in Soil Extracts Using 4-g. Carbon Columns and Special 8-g. Carbon Columns

Introduction

Several experiments were performed to study the effect of different amounts of carbon in the chromatographic columns on the sorption of organic phosphorus from soil extracts. Invariably, more phosphorus was recovered from the columns which contained the larger amounts of carbon. In this experiment the recovery of organic phosphorus from soil extract by regular 4-g. carbon columns was compared to the recovery of

the organic phosphorus from specially designed chromatographic columns which provided more favorable sorptive conditions. The purpose of the comparison was to evaluate the sorptive efficiency of the regular 4-g. carbon columns.

Procedure

Two sets of chromatographic columns were prepared, each set consisting of nine columns. One set was prepared with 4 g. of carbon in the regular Pyrex tubing having 1.6 cm. inside diameter. The 4 g. of carbon made an adsorbent bed about 5 cm. in height. The second set of nine columns was designed to provide more favorable conditions for sorption of organic phosphorus. Long slender Pyrex tubes having 1.0 cm. inside diameter were prepared with 8 g. of carbon. In the special columns the carbon bed was over 20 cm. in height.

Samples of Harpster silty clay loam F-2868 were extracted by the new extraction procedure. An inorganic phosphorus solution was made up in 0.5 N HCl which had an inorganic phosphorus concentration similar to that of the soil extract, 18 µg. of phosphorus per 10 ml. Within each set of nine columns, five columns received a 10-ml. aliquot of soil extract, and four columns received a 10-ml. aliquot of the inorganic phosphorus solution. All columns were eluted with 450 ml. of 0.5 N HCl. The phosphorus content of the ashed carbon was determined. The organic phosphorus content of the

soil extract was determined by the method of difference also.

Results and discussion

The experimental results are presented in Table 22. The organic phosphorus content of 10 ml. of soil extract was 15.82

Table 22. Determination of organic phosphorus in soil extract by regular 4-g. carbon columns, by special 8-g. carbon columns, and by the method of difference

Method of analysis	Organic phosphorus per 10 ml. of soil extract, μg.	Variance of organic phosphorus
Regular 4-g. carbon columns	15.82	0.00312
Special 8-g. carbon columns	16.08	0.01248
Difference	16.19	0.07104

μg. of phosphorus determined with 4-g. carbon columns, 16.08 μg. of phosphorus determined with the special 8-g. carbon columns, and 16.19 μg. of phosphorus determined by the method of difference. The organic phosphorus value calculated by the method of difference was not significantly different from the value obtained with the special 8-g. columns. The 8-g. carbon columns designed to provide more favorable conditions for the sorption of organic phosphorus produced a higher value for organic phosphorus than the regular 4-g. carbon

columns at a probability level between 0.10 and 0.05. The organic phosphorus sorbed by the regular 4-g. columns was 98.4% of the amount sorbed on the special columns. In absolute units, the difference between the two types of columns was about 0.25 μg. of phosphorus. The difference in results provided by the two types of columns was statistically significant in this experiment only because the precision of the analyses was high. Had the experimental errors associated with the two types of chromatographic determinations been larger, the difference in results would not have been significant.

The comparison of the chromatographic determinations of organic phosphorus by the regular 4-g. carbon columns with the special 8-g. carbon columns indicates that the chromatographic procedure with the regular 4-g. carbon columns is satisfactory. The regular chromatographic columns sorbed almost as much organic phosphorus as the special columns. The 4-g. chromatographic columns have the advantages of requiring a smaller blank value and permitting more rapid elution.

The Effect of HCl Normality of the Soil Extract and of the Eluant on the Sorption of Organic Phosphorus from Soil Extract and on the Elution of Inorganic Phosphorus from the Carbon Columns

Introduction

In the original procedure for the separation of organic phosphorus from soil extracts by sorption on activated carbon, Goring (1955) reported that the alkali extract was acidified to an HCl normality of 0.5 before addition of the activated carbon. Goring did not elaborate on the effect of acid strength. The results of a preliminary experiment on the effect of the HCl normality of inorganic phosphorus solutions on the retention of inorganic phosphorus by carbon, reported in a previous section, showed that the least amount of inorganic phosphorus was retained on carbon from solutions that were 0.5 N in HCl and the maximum amount was retained from distilled water solutions. This experiment was conducted to investigate the effect of acid strength of soil extract and eluant on the chromatographic determination of organic phosphorus. Three acid strengths were studied: 0.1, 0.5, and 1.0 N HCl.

Procedure

Samples of Ida silt loam were extracted with 1 N HCl and 0.5 N NH_4OH as in the new extraction procedure except that the combined extract was not treated with 5 ml. of con-

centrated HCl. Instead, the combined extract was adjusted to an acid strength 0.1 N in HCl. Three 150-ml. portions of the extract were treated with HCl and adjusted to a final volume in such a manner that the three extracts contained identical concentrations of inorganic and organic phosphorus but had three different HCl normalities, 0.1, 0.5, and 1.0. Three inorganic phosphorus solutions containing 1000 μg. of phosphorus per 10 ml. were prepared in 0.1, 0.5, and 1.0 N HCl.

Ten-milliliter aliquots of inorganic phosphorus solutions were added to regular 4-g. carbon columns. Other columns received 20 ml. of soil extract and 10 ml. of the inorganic phosphorus solution, both of the same HCl normality. Thus, all columns received 1000 μg. of inorganic phosphorus, and some of the columns received soil extract in addition. All columns were eluted with 300 ml. of HCl of an appropriate normality. Consequently, all the additions, inorganic phosphorus solution, soil extract, and HCl eluant, applied to a given column were of the same HCl normality, either 0.1, 0.5, or 1.0. The ashed carbon was analyzed for phosphorus. The experiment was repeated using samples of Harpster silty clay loam F-2868.

Results and discussion

The experimental results for both soils are given in Table 23. The effect of the HCl normality is evident on the

Table 23. Effect of HCl normality of soil extract, inorganic phosphorus solution, and eluant on the sorption of organic phosphorus and retention of inorganic phosphorus in chromatographic columns

HCl normality of the phosphorus solution, soil extract, and eluant	Average phosphorus content of ashed carbon, μg.		Sorbed organic phosphorus, μg. (Difference between columns 2 and 3)	Variance of organic phosphorus
	With extract	Without extract		
(Extracts of Ida silt loam)				
0.1	18.34	6.14	12.20	0.00480
0.5	16.86	5.02	11.84	0.02138
1.0	16.38	4.82	11.56	0.03226
(Extracts of Harpster silty clay loam)				
0.1	40.20	6.33	33.87	0.81610
0.5	38.58	6.35	32.23	0.93168
1.0	37.52	4.71	32.81	0.18213

sorption of organic phosphorus as well as on the retention of inorganic phosphorus for both soils although the differences were not statistically significant in the case of the extracts from Harpster silty clay loam. For the extracts from Ida silt loam, the increase in HCl normality from 0.1 to 1.0 reduced the apparent sorption of organic phosphorus from 12.20 to 11.56 μg. of phosphorus per column, a reduction that was significant at a probability level of about 2%. This increase in normality caused a corresponding reduction in the amount of

retained inorganic phosphorus from 6.14 to 4.82 μg. of phosphorus per column. The largest effect of the HCl normality on the organic phosphorus determination and on inorganic phosphorus retention was between 0.1 and 0.5 N. The apparent reduction of sorbed organic phosphorus caused by this change in normality was significant at about the 6% level.

One possible explanation for the behavior of the inorganic phosphorus in the chromatographic columns might be that only ionized phosphoric acid is retained by the carbon and that with increasing acid strength of the medium the ionization of the phosphoric acid is suppressed. In the case of soil extracts, the change in acidity between 0.1 and 0.5 N might conceivably be associated with a difference in precipitation of inorganic phosphate compounds in the carbon columns. To obtain clear evidence as to the nature of the apparent change of organic phosphorus retention with degree of acidity would probably require tagging of the inorganic phosphorus with P^{32}.

The Sorptive Capacity of 4-g. Carbon Columns for Organic Phosphorus from a Soil Extract

Introduction

Many of the investigations reported earlier were concerned with the quantitative recovery of organic phosphorus from aliquots of soil extract added to the chromatographic columns. The aliquot size varied from 10 to 25 ml. The

separation of organic phosphorus from aliquots of soil extract by chromatographic columns containing 4 g. of carbon was satisfactory. The experiments reported, however, did not provide information on the capacity of 4 g. of carbon for organic phosphorus. The purpose of this experiment was to determine the total sorptive capacity of the chromatographic columns.

Procedure

Several samples of Harpster silty clay loam F-2868 were extracted by the new method, and the extracts were combined to form a large, uniform supply. The suspended solid material was allowed to flocculate and settle, and only the supernatant was used. An inorganic phosphorus solution was prepared in 0.5 N HCl and 0.25 N NH_4Cl which had approximately the same inorganic phosphorus concentration as the soil extract, 18 μg. of phosphorus per 10 ml.

Eighteen 4-g. carbon columns were prepared. Increasing volumes of soil extract (supernatant) were added to the columns. The inorganic phosphorus solution was also added to the columns either alone or in combination with the soil extract in such quantity that all columns received the same total quantity of inorganic phosphorus, 180 μg. All columns were eluted with 300 ml. of 0.5 N HCl. The phosphorus content of the ashed carbon was determined.

Results and discussion

The results of the experiment are presented in Figure 1. The phosphorus content of the ashed carbon is shown on the vertical axis, and the volume of added soil extract (supernatant) is shown on the horizontal axis. The relationship between phosphorus content in the ashed carbon and the volume of added soil extract is linear over the range of volumes studied in the experiment. Either all of the organic phosphorus in the added extract was sorbed on the carbon, or a constant proportion of added organic phosphorus was lost from the columns. In either case the maximum sorptive capacity of the carbon could not be determined from the results.

Only the supernatant of the soil extract was used in this experiment. The organic phosphorus concentration of the the supernatant was 7.0 μg. phosphorus per 10 ml. as compared to a concentration of about 16 μg. of organic phosphorus per 10 ml. of whole soil extract (supernatant plus solids). Hence, the experiment was concerned with the sorption of soluble organic phosphorus only. The largest addition of supernatant resulted in the sorption of about 70 μg. of phosphorus. If the treatment had consisted of the addition of whole extract rather than supernatant only, the amount of organic phosphorus involved would have been about 160 μg. Obviously the separation of the organic phosphorus contained in the solid material of the whole extract is a matter of

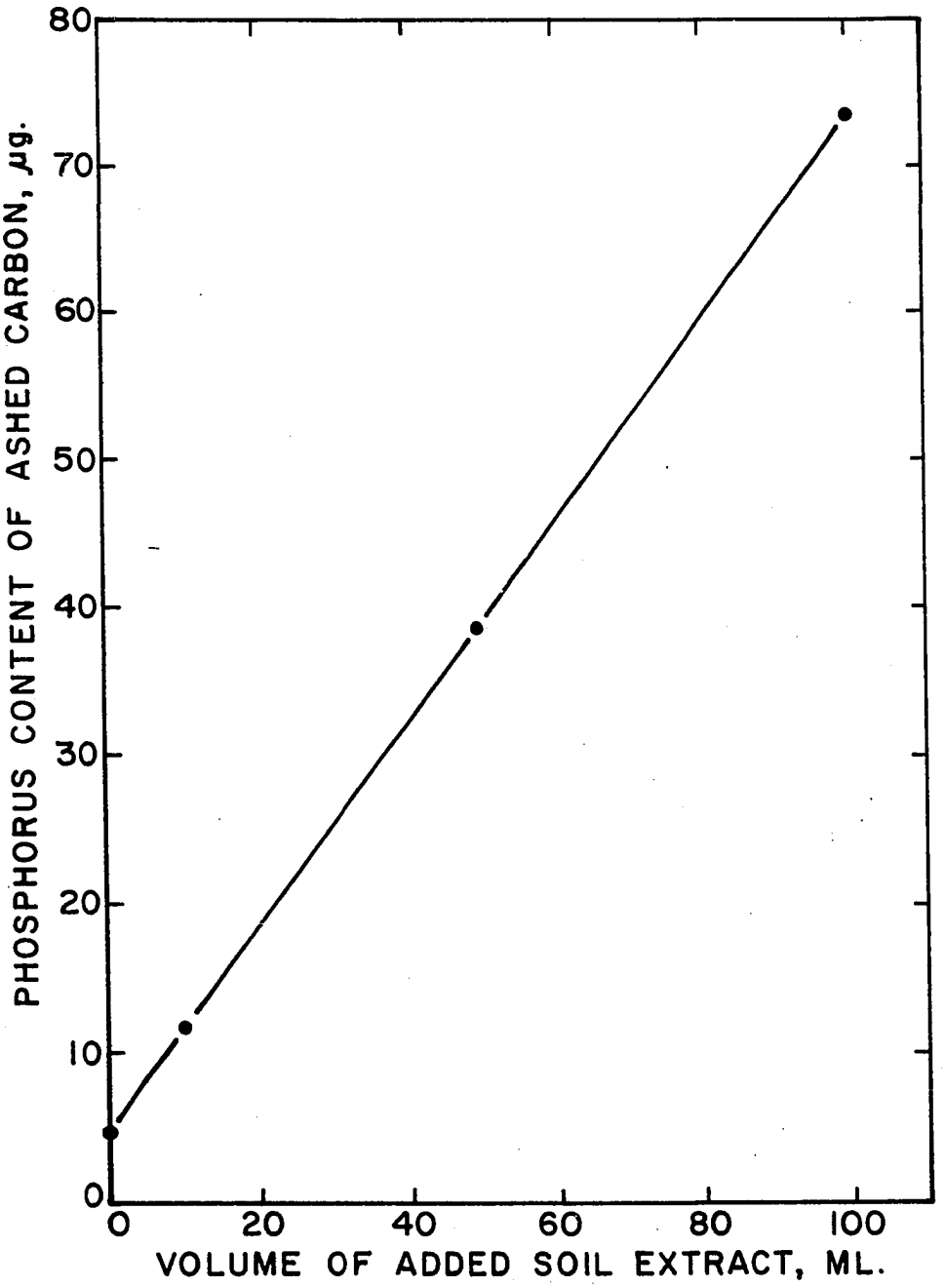

Figure 1. Retention of phosphorus on 4 g. of carbon from columns treated with different quantities of soil extract

mechanical filtration, a process that is probably independent of the physical and chemical sorption of the soluble organic phosphorus on the carbon surfaces. Thus it can be assumed that the linear relationship between recovered phosphorus in the ashed carbon and added volume of supernatant would also apply for added whole soil extract up to a volume of 100 ml. Of course, the slope of the line would be greater with the whole extract than with the supernatant because the whole extract would have a higher concentration of total organic phosphorus.

The soil used in this experiment, Harpster silty clay loam F-2868, had the highest content of organic phosphorus of any soil studied by Van Diest (1957). In the chromatographic determination of organic phosphorus, 20 ml. of whole soil extract is usually added to the 4-g. carbon columns. This volume of extract and organic phosphorus content are well within the range of conditions covered by this experiment.

Comparison of Methods of Organic Phosphorus Determination

Introduction

The organic phosphorus content of four soil extracts was determined by the chromatographic method and by the method of difference. Two acid and two alkaline soils were selected for extraction by the new extraction procedure. The individual

analyses were replicated to provide an estimation of the precision of the two methods.

Procedure

Samples of four soils were extracted by the new extraction procedure using 1 N HCl and 0.5 N NH_4OH. Two of the soils were calcareous, Harpster silty clay loam (F-2872) and Ida silt loam (F-2882). Two of the soils were acid, Nicollet loam (F-2887) and Shelby loam (F-2902). A brief description of the soils is given in a previous section. Four analyses by both methods of organic phosphorus determination were made on each of the soil extracts. For the method of difference a 25-ml. aliquot of extract was taken for total phosphorus determination, and a 10-ml. aliquot was taken for inorganic phosphorus determination. Twenty 4-g. carbon columns were prepared. The four columns which served as blanks received a 20-ml. aliquot of an inorganic phosphorus solution which contained 36 μg. of phosphorus to simulate the effect of the inorganic phosphorus content of the soil extracts on the chromatographic determination of organic phosphorus. The inorganic phosphorus solution was prepared in 0.5 N HCl and 0.25 N NH_4Cl. The other columns received 25-ml. aliquots of soil extract. All columns were eluted with 300 ml. of 0.5 N HCl.

Values of organic phosphorus were calculated by both

methods for each of the soil extracts. The variance associated with each value of organic phosphorus was also calculated. The results of the two methods were compared by means of a t-test.

Results and discussion

The values of organic phosphorus as determined by the two methods and the variances associated with those values are presented in Table 24. The results of the two methods of analysis were compared by means of a t-test for each of the soil extracts, and the probability values associated with each of the calculated t-values are also listed in the table. The two methods of organic phosphorus determination gave significantly different results for three of the four soil extracts. The chromatographic method gave significantly higher values for organic phosphorus for two of the soil extracts and a significantly lower value for the third soil extract than did the method of difference. The results obtained in this comparison with four soils probably do not represent a typical cross-section of results that might be expected with soils in general because the soils were selected to illustrate the differences between methods of analysis.

The size of the variances reflects the precision of the analytical methods. The precision of the chromatographic method is clearly superior to that of the method of differ-

Table 24. Comparison of methods of determining organic phosphorus in four soil extracts

Soil no.	Chromatographic method			Method of difference			Probability level from t-table
	Organic phosphorus, µg./25 ml.	Variance of organic phosphorus	Coefficient of variation, %	Organic phosphorus, µg./25 ml.	Variance of organic phosphorus	Coefficient of variation, %	
F-2872	29.07	0.00296	0.19	29.48	0.01772	0.45	0.025
F-2882	15.29	0.05685	1.51	10.29	0.30713	5.35	0.01
F-2887	19.00	0.00240	0.26	17.85	0.05572	1.32	0.01
F-2902	8.36	0.00448	0.80	8.35	0.03327	2.18	0.50

ence. The largest experimental error of both methods of determination was with Ida silt loam F-2882. This may be coincidental, or it may be a function of some trait peculiar to that soil extract.

This experiment does not establish the superiority of either method but merely indicates that with some soils the two methods give definitely different values. Further work is needed to establish which, if either, of the methods yields the more accurate values.

SUMMARY

A method of determining the organic phosphorus content of soil extracts was developed which is capable of precise measurements of organic phosphorus even in solutions containing large quantities of inorganic phosphorus. The method involves a chromatographic separation of total organic phosphorus from the soil extract by sorption of the organic phosphorus compounds on an adsorbent bed consisting of 4 g. of Norit-A decolorizing carbon. Before use, the carbon is washed successively with concentrated HCl, 95% alcohol, 6 N NH_4OH, and concentrated HCl. Inorganic phosphorus is eluted from the columns with 0.5 N HCl. The carbon and sorbed organic phosphorus are extruded from the column and ashed at 500°C. The phosphorus content of the ashed carbon is determined colorimetrically. The quantity of sorbed organic phosphorus is calculated from the difference between the phosphorus content of the ashed carbon from columns which have received soil extract and columns which have received a solution containing a quantity of inorganic phosphorus equivalent to that added in the soil extract.

The chromatographic columns failed to separate quantitatively the organic phosphorus from NaOH extracts of soils. The chromatographic separation of organic phosphorus from NH_4OH extracts, however, appeared to be essentially complete. Virtually no organic phosphorus could be detected in the 0.5

N HCl eluate from columns which had received NH_4OH extracts.

The completeness of the chromatographic separation of organic and inorganic phosphorus was studied by the technique of isotopic dilution after tagging the inorganic phosphorus of a soil extract with P^{32}. The inorganic phosphorus retained was found to be less than 1% of the total phosphorus content of the ashed carbon with the particular soil extract used in the experiment. The inorganic phosphorus sorbed by the carbon from the soil extract accounted for only a negligible part of the approximately 30% higher value for organic phosphorus obtained on this extract by the chromatographic method than by the traditional method of difference. The method of difference involves measuring the total and inorganic phosphorus in the extract colorimetrically and subtracting the latter from the former to obtain a figure for organic phosphorus.

The chromatographic determination of organic phosphorus in soil extracts was found to be independent of the concentration of inorganic phosphorus in the extract, provided the blank columns are treated with the same amount of inorganic phosphorus as the columns which received the soil extract.

The apparent values for organic phosphorus determined chromatographically varied somewhat with the HCl normality of the soil extract and eluant. Slightly higher values were obtained from columns which received extract and eluant in 0.1 N HCl than from columns treated with extract and eluant

in 1.0 N HCl. A slightly larger quantity of inorganic phosphorus was retained by the columns from 0.1 N HCl systems.

The value of organic phosphorus determined chromatographically appeared to increase with the amount of carbon used in the columns. Although 10-g. carbon columns consistently provided slightly higher values of organic phosphorus than did 4-g. columns, the difference in results was not significant.

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